HISTOLOGICAL AND MOLECULAR SUBTYPING OF MEDULLOBLASTOMA USING IMMUNOHISTOCHEMICAL MARKERS

A DISSERTATION SUBMITTED IN PART

FULFILLMENT OF THE REGULATION FOR THE

AWARD OF THE DEGREE OF M.D. PATHOLOGY

BRANCH III

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CERTIFICATE

This is to certify that the thesis entitled "Histological and Molecular subtyping of Medulloblastoma using immunohistochemical markers" submitted by Dr. Magesh P., in partial fulfilment of the requirement for the M.D. Branch III (Pathology) Degree Examination of the Tamil Nadu Dr. M.G.R. Medical University, Chennai, to be held in April 2016, is the bonafide work done by him.

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Could you please sign the agreement and send it to Dr. Nihal Thomas, Addl. Vice Principal (Research), so that the grant money can be released.

With best wishes,

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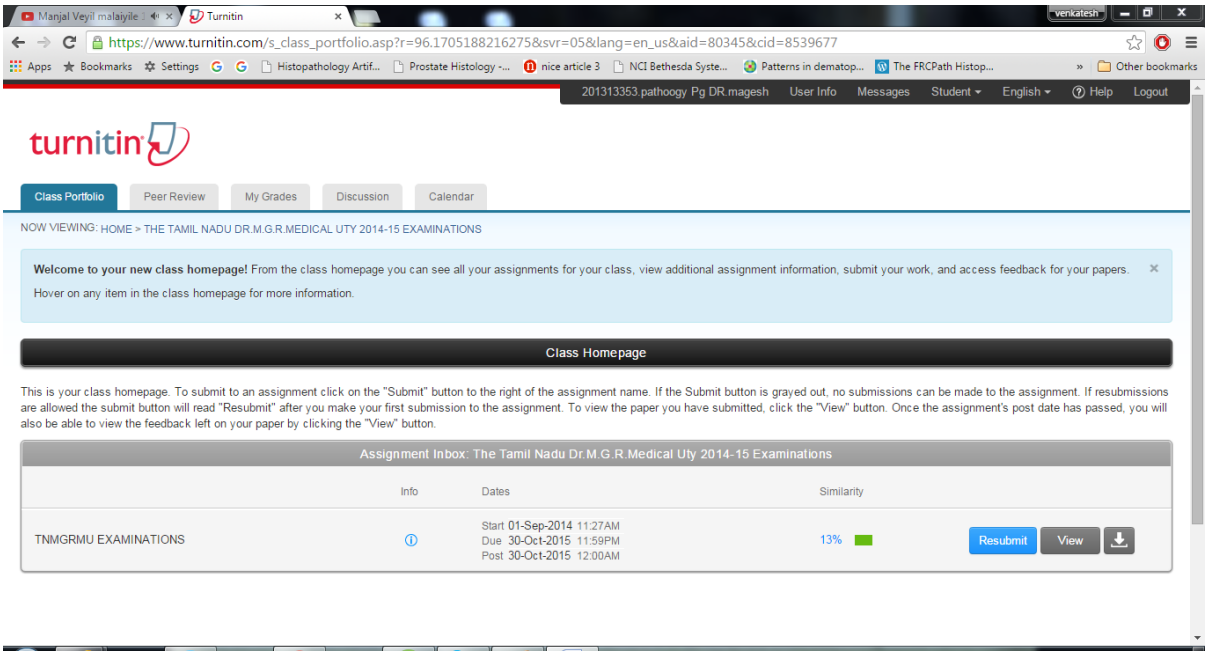
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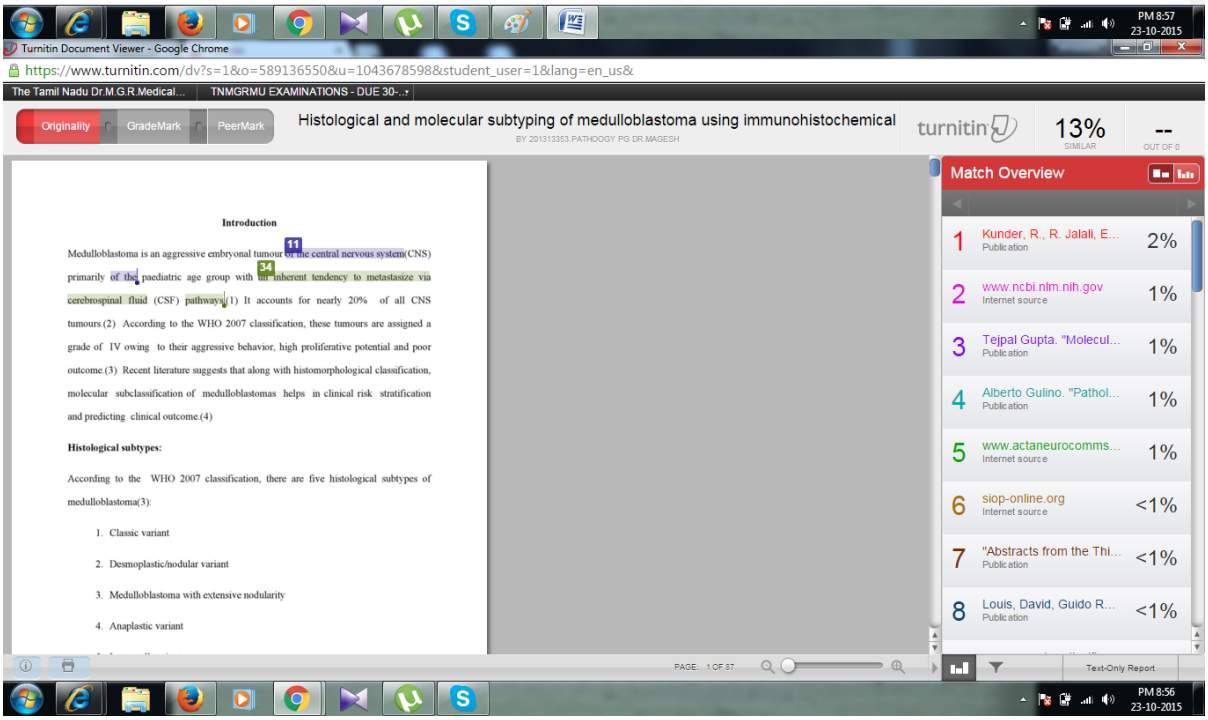
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Introduction
Medulloblastoma is an aggressive embryonal tumour of the central nervous system(CNS) primarily of the paediatric age group with an inherent tendency to metastasize via cerebrospinal fluid (CSF) pathways.(1) It accounts for nearly 20% of all CNS tumours.(2) According to the WHO 2007 classification, these tumours are assigned a grade of IV owing to their aggressive behavior, high proliferative potential and poor outcome.(3) Recent literature suggests that along with histomorphological classification, molecular subclassification of medulloblastomas helps in clinical risk stratification and predicting clinical outcome.(4)
Histological subtypes:
According to the WHO 2007 classification, there are five histological subtypes of medulloblastoma(3):
1. Classic variant
2. Desmoplastic/nodular variant
3. Medulloblastoma with extensive nodularity
4. Anaplastic variant
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ABBREVIATIONS

AXIN	Axis Inhibition of Wnt Pathway
APC	Adenomatous polyposis coli
BCL2	B cell lymphoma 2
BCLxl	B cell lymphoma xL
CNS	Central nervous system
CSF -	Cerebrospinal fluid
CTNNB1	Catenin (Cadherin-Associated Protein), Beta 1
CDK6-	Cyclin-dependent protein kinase
DKK1 -	Dickkopf WNT signaling pathway inhibitor 1
DSH	Disheveled
ERBB2	Avian erythroblastosis oncogene B
EGL	External Granular Layer
FLI A	Friend leukemia virus integration A
FGF	Fibroblast growth factor
GAB-1	Growth factor receptor-bound protein 2 associated-binding protein 1
GSK-3Beta	Glycogen synthase kinase-3Beta
GNP	Granule neuronal precursors
GRM8	Glutamate receptor metabotropic 8
HDAC 5	Histone Deacetylase 5
JAG	Jagged
KDM6A	Lysine (K)-Specific Demethylase 6A)
KCNA	Potassium channel gene
LEF	Lymphoid enhancer factor

MAX	myc-associated factor X
MBEN	Medulloblastoma with extensive nodularity
MYC L	Avian MC29 myelocytomatosis
MYCN	Avian MC29 myelocytomatosis
MIB-1	Mind Bomb
MRI	Magnetic resonance imaging.
NPR-3	Natriuretic Peptide Receptor 3
OTX2	Orthodonticle
PNET	Primitive Neuroectodermal tumour
PTCH	Patched 1
SHH-	Sonic Hedgehog
SFRP1	Secreted frizzled-related protein 1 (SFRP1)
SMO	Smoothened
SUFU	Supressor of fused homolog
TCF	T-cell transcription factors
TSG APC	Tumour suppressor gene Adenomatous Polyposis coli.
VEGF	Vascular Endothelial Growth Factor
WNT	Drosphila melanogaster wingless gene.
WIF1	WNT-inhibitory factor 1.
YAP-1	Yes associated protein.

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ABSTRACT

TITLE OF THE ABSTRACT : Histopathological and molecular subtyping of medulloblastoma using immunohistochemical markers.

DEPARTMENT : General Pathology

NAME OF THE CANDIDATE : Dr. Magesh.P

DEGREE AND SUBJECT : M.D., PATHOLOGY

NAME OF THE GUIDE : Dr. Geeta Chacko

OBJECTIVES:

To study the demographic, histological features and expression profile of the immunohistochemical markers β-catenin, GAB-1 and NPR-3 in adult and paediatric medulloblastomas and to correlate the expression of these three markers with the histological subtypes of medulloblastoma.

METHODS:

One hundred and thirteen cases of medulloblastoma were included in this study. A detailed review of the H&E stained slides and previous immunohistochemical studies was done. Immunohistochemistry for β-catenin, GAB-1 and NPR-3 were performed on all 113 cases. Correlation between patient demographic profile, morphological subtypes and immunohistochemical markers was done.

RESULTS:

Medulloblastomas are primarily tumors of childhood and 77% of the study population was children. The mean age at diagnosis of medulloblastomas was 13 years. In children there was a clear male preponderance, whilst in adults there was a near equal gender distribution. Medulloblastomas occurred predominantly in the midline with only a quarter arising laterally in the cerebellar hemisphere. The predominant histological subtype corresponded to the Classic variant. Desmoplastic medulloblastomas formed the next major histological variant, followed by the Large Cell variant. Anaplastic and Medulloblastoma with extensive nodularity (MBEN)

subtypes formed ≤5% of medulloblastomas. Medulloblastomas in the cerebellar hemispheres were predominantly of the Desmoplastic variant. WNT signaling activation was seen in 44.2% of medulloblastomas, and in the majority of cases with Classic histology 65.7%. Shh signaling activation was seen in 23% of medulloblastomas, predominantly in the desmoplastic variant. The non WNT/Shh sub-group constituted 34.5% of medulloblastomas.

Keywords : medulloblastoma, β-catenin, desmoplastic, cerebellar hemisphere.

INTRODUCTION

Medulloblastoma is an aggressive embryonal tumour of the central nervous system(CNS) primarily of the paediatric age group with an inherent tendency to metastasize via cerebrospinal fluid (CSF) pathways.(1) It accounts for nearly 20% of all CNS tumours.(2) According to the WHO 2007 classification, these tumours are assigned a grade of IV owing to their aggressive behavior, high proliferative potential and poor outcome.(3) Recent literature suggests that along with histomorphological classification, molecular subclassification of medulloblastomas helps in clinical risk stratification and predicting clinical outcome.(4)

Histological subtypes:

According to the WHO 2007 classification, there are five histological subtypes of medulloblastoma(3):

1. Classic variant
2. Desmoplastic/nodular variant
3. Medulloblastoma with extensive nodularity
4. Anaplastic variant
5. Large cell variant

Classic variant:

Classic variant has closely packed round to oval shaped cells with hyperchromatic nuclei surrounded by scant cytoplasm. Neuroblastic rosettes (Homer Wright rosettes) are observed in 40% of cases.

Desmoplastic /nodular variant:

Desmoplastic variant is characterized by nodular reticulin free zones (pale islands) surrounded by densely packed highly proliferative cells.

Medulloblastoma with extensive nodularity:

This was previously described as cerebellar neuroblastoma and has expanded lobular architecture due to the fact that reticulin free zones become unusually enlarged and rich in neuropil like tissue. Internodular areas are markedly reduced in some areas.

Anaplastic variant:

Increased nuclear pleomorphism, nuclear moulding, cell-cell wrapping and high mitotic activity characterise this variant of medulloblastoma. Apoptosis is prominent in this variant. Presence of these features in focal areas is not sufficient.

Large cell medulloblastoma:

2-4% of medulloblastomas come under the large cell variant and comprise monomorphous population of cells with large, round, vesicular nuclei, prominent nucleoli and variable amount of cytoplasm. Mitotic and apoptotic figures are abundant.(5)

Molecular sub grouping of medulloblastoma:

Based on genetic analysis using mRNA based expression analysis and DNA based copy-number analysis as well as whole genome sequencing studies, gene expression signatures for medulloblastoma have been identified that can predict patients survival with more accuracy than clinical risk stratification.(6)

There are four molecular subtypes based on recent literature.(7)

1. WNT subtype.
2. SHH subtype.
3. Group 3.
4. Group 4.

WNT subtype:

WNT- Wingless signaling pathways. These are proteins which activate the transmembrane frizzled receptors which locks the β-catenin from binding with APC,

Axin 1 and 2, caesine kinase1, GSK-3B. As a result β-catenin is not degraded and is translocated into nucleus activating the cyclin D and C-myc.(8, 9)

Recent studies by Min et al(7) have shown that medulloblastomas with activation of the WNT signaling pathway affected the older children, showed a classic histology with nuclear expression of β-catenin, CTNNB1-mutation, monosomy 6 and had a relatively good prognosis.

SHH subtype:

During normal Sonic hedgehog pathway (SHH) signalling, SHH ligand binds to PTCH 1, which releases receptor smoothened (SMO) from inhibition which in turn activates the Gli transcription factor and transcription occurs in the nucleus. So in medulloblastoma loss of PTCH and mutation in SMO or Sufu causes accumulation of Gli transcription factor. Min et al(7), found medulloblastoma with SHH pathway in infants and young adults was associated with the desmoplastic variant, GAB-1 expression, PTCH deletion and an intermediate prognosis.

Group 3 and 4:

Group 3 medulloblastomas are frequently seen in infants and children with classic /anaplastic histology with poor prognosis.(7) Group 4 patients with intermediate prognosis showed a classic or large cell/anaplastic histology. Genetic studies revealed dysregulation of histone methylation occurs in group 3 and group 4. Additionally,

mutation in KDM6A family showed dysregulation of lysine demethylation in a subgroup 3 and 4.(8)

Use of immunohistochemical markers in molecular sub classification:

Immunohistochemical (IHC) markers have been identified that correspond to certain specific genetic signatures seen in medulloblastomas. Use of these IHC markers has been reported to help in sub grouping of medulloblastomas.

Ellison et al, found four immunohistochemical markers to aid in molecular stratification. β-catenin was very useful in identifying tumours with WNT signaling pathway defects with almost 98% specificity. Cytoplasmic positivity for GAB-1 was seen in internodular areas of desmoplastic variants and therefore useful in identifying tumours with defect in SHH pathways. FLI A and YAP-1 negative expression was reported to be markers of non-WNT/SHH pathway.(10) The expression of immunomarkers is as shown in the table below (Table 1).

Table 1: Immunoexpression profile used for molecular subgrouping of medulloblastomas.

Molecular Group	**Immunoreactivity**			
	GAB1	**β – catenin**	**Fillamin A**	**YAP1**
SHH	Cytoplasmic	Cytoplasmic	Cytoplasmic	Nuclear Cytoplasmic
WNT	Negative	Nuclear Cytoplasmic	Cytoplasmic	Nuclear Cytoplasmic
Non SHH/WNT	Negative	Cytoplasmic	Negative	Negative

Although few studies established clinical correlation with risk stratification based on these markers, few other studies found a fair degree of overlap between histological and molecular subtypes. (11)

In more recent years, Northcott et al(12), used another set of four markers, namely DKK1, SFRP1, NPR-3 and KCNA 1 for molecular sub-classification of medulloblastoma based on the results of genome wide DNA copy number and mRNA expression profiles

and found that these markers reliably classified medulloblastomas into four subclasses, DKK1 (WNT), SFRP1 (SHH), NPR3 (group C) and KCNA1 (group D).

However, Min et al(7) who found that while nuclear β-catenin and GAB-1 easily identified WNT and SHH groups without any significant overlap use of NPR3 and KCNA1 did not appear to be either specific or sensitive for genetic sub grouping.

Kool et al(13) performed a meta-analysis of molecular and clinical data on 550 medulloblastomas from seven independent studies. Their data demonstrated that medulloblastoma is not a single disease and that the four major subgroups are transcriptionally, genetically, demographically, clinically and prognostically distinct.

The meta-analysis also suggested that there are probably subsets within each subgroup.

The prognostic factors like metastatic stage, histology, and MYC and MYCN amplification performed differently in different subgroups. For example, MYC and MYCN amplification were found to predict an unfavorable outcome in the entire cohort of medulloblastomas. However, MYC amplification was not prognostic in Group 3 and in contrast MYCN amplification was prognostic in SHH and Group 4 medulloblastomas. This meta-analysis emphasized the need for identifying prognostic markers which would serve as prognostic for each subgroup.

There is a consensus amongst investigators that there are four main subgroups of medulloblastomas with demographic, transcriptional, genetic and clinical differences that have important clinical implications(Table 2).(14)

Table 2: Features of molecular subgroups of medulloblastoma.

Molecular subtypes	Age	Gender M:F	Metastasis	Histology	Genetic/gene expression
WNT Subgroup	Children followed by adults	2:2	Rarely metastasize	Classic, rarely large cell histology	CTNNB1 mutation, Monosomy 6,WNTsignaling and MYC expression
SHH subgroup	Commonly Infants	2:2	Uncommonly metastasize	Desmoplastic, classic, LCA and MBEN	PTCH/SUFU/SMO mutations, 9qdeletion, MYC N amplification
Group 3	Commonly Children	2:1	Very frequently metastasize	Classic and large cell morphology	MYC amplification, photoreceptor/GABAergic pathways
Group 4	Children and infants	2:1	Frequently metastasize	Classic and large cell morphology	CDK6 amplification, neuronal, glutamatergic pathways, minimal MYC family expression

There are limited studies from the Indian subcontinent. Das et al(15) in a retrospective study performed immunohistochemistry on 30 cases of medulloblastoma using MIB-1, c-MYC, ERBB2, bcl2 and bclxL. They found that while bcl-2, ERBB2 and MIB-1 were potential markers of aggressive behavior, the protein expression patterns did not correlate with histological subtypes and that c-MYC expression did not correlate with progression free survival.

Kunder et al (16) have done microRNA profiling based on the 9 miRNAs and 12 protein coding genes and found this to have an overall accuracy of 97%. In this study, age at

diagnosis, histology, gender and relative survival rates were found to be similar in the medulloblastomas reported from the American and European subcontinents.

The purpose of our study is to carry out a detailed histological and immunohistochemical study of adult and paediatric medulloblastomas using immunohistochemical markers GAB-1 and β-catenin and determine the correlation of these two markers with the histological subtypes.

AIMS

1. To study the histological features of adult and paediatric medulloblastoma and classify them into the histological subtypes as defined by WHO.

2. To study the expression profile of the immunohistochemical markers β-catenin, GAB-1 and NPR-3 in medulloblastomas.

3. To correlate the expression of the three markers, β-catenin, GAB-1 and NPR-3, with the histological subtypes of medulloblastoma.

REVIEW OF LITERATURE

Central nervous system (CNS) tumors are the second most common group of cancers in childhood next to leukemia(17) and the most common solid malignancies of childhood. Gliomas constitute about 50% of the childhood brain tumors with astrocytomas forming the predominant subtype and are the most common brain tumour in adults. Age distribution shows that primitive neuroectodermal tumours and ependymomas are common in children less than 10 years. (17)

Medulloblastomas are the most common paediatric malignant brain tumours constituting approximately 20% of childhood brain tumors.(18)(19) They are rare in adults, contributing to less than 1% of all adult CNS tumours and are usually located in the cerebellar hemsipheres due to migration of undifferentiated cells from the posterior medullary velum to cerebellar hemispheres.(20)

In the WHO 2000 classification five entities were defined as belonging to the undifferentiated neuroepithelial tumor category namely, medulloblastoma, supratentorial PNET, medulloepithelioma, ependymoblastoma and atypical teratoid /rhabdoid tumour.(21) In the WHO 2007 classification, medulloblastoma was identified as separate category of embryonal tumour with five different histological subtypes. This was based on morphological, immunohistochemical, molecular, clinical and biological profiles.(3)

Medulloblastomas are small round blue cell tumours with a propensity to metastasize via cerebrospinal pathways, resulting in leptomeningeal dissemination as an initial diagnosis in 25-30%. They have an aggressive behaviour and contribute to a substantial number of deaths as a result of tumour progression. Medulloblastomas are therefore considered as WHO Grade IV tumours for their aggressive behaviour, despite chemotherapy, radiotherapy and surgery(22) and because of their high proliferative potential.

INCIDENCE, AGE AND GENDER DISTRIBUTION

Medulloblastomas account for approximately 6% of all intracranial tumours.(23) Children are 4.6 times more prone for primitive neuroectodermal tumours than adults.(23) Among primitive neuroectodermal tumours, medulloblastoma is the most common, comprising about 95% of PNETs and have an incidence of approximately 0.5 per 100000 in children less than 15 years of age. However in children less than 2 years of age, astrocytomas and ependymomas are more frequent than medulloblastomas.

In a recent multi-institutional study from India, paediatric CNS tumors accounted for 10-21% of all CNS tumors. Medulloblastomas along with other embryonal tumours comprised the second largest group following glioma, with an average prevalence of 22.4% of paediatric brain tumors.(24) In a retrospective study from this institution covering over 14 years the incidence of medulloblastoma was found to be 11.3%.(25)

In children, medulloblastomas have a bimodal age distribution, with peaks between 3-4 years and 8-9 years. In adults the incidence of medulloblastomas are high in the age

group 15 to 19 years and decreases with increasing age. (10) Most centers consider patients upto 18 years as pediatric. Age cut-off of less than or equal to 3 years plays a significant role in risk stratification.(26) Childhood medulloblastomas are more common in male than females (1.5:1). In infants, medulloblastomas may occur as part of familial cancer syndromes .(11)

SITE & HISTOGENESIS (12):

Medulloblastomas occur at two sites.

1. Lateral cerebellar hemispheres.

   The external granular layer containing stem cells and pluripotent cells of the ventricular subependymal matrix are the cells of origin for the desmoplastic variant of medulloblastoma.

2. Cerebellar vermis.

   The subtypes of medulloblastomas that arise from the cerebellar vermis, originate from the ventricular matrix and Purkinje neurons.

The existence of different histological subtypes of medulloblastomas suggests that different cells of origin and different signaling pathways are involved in their molecular sub grouping. Those that arise from the granule neuron precursor (GNP) cells have aberrant activation of the sonic hedgehog pathway (Shh). The subgroup of medulloblastomas with the Wingless signaling (WNT) pathway activating mutation are

found to arise from outside the cerebellum, mainly from the lower rhombic lip and embryonic dorsal brainstem instead of the developing cerebellum.(27)

There is evidence that medulloblastomas can arise from the granule precursor neuronal cell in the cochlear nuclei.(28)

CLINICAL AND RADIOLOGICAL FEATURES

Most patients with medulloblastoma present with symptoms of increased intracranial pressure due to the blockage of the cerebrospinal pathway in the fourth ventricle. Headache, vomiting, lethargy and drowsiness are the usual presenting symptoms. Vermian compression and infiltration presents as ataxia while brainstem infiltration and compression can cause cranial nerve palsies. (29)

Medulloblastoma can be diagnosed using standard neuroimaging techniques. On magnetic resonance imaging (MRI), the tumour is visible as a heterogeneously hypo-intense lesion on T1w images, hyperintense on T2w images, and restricted diffusion on the DTI images indicating increased cellularity. They typically enhance well with contrast and may have cysts with or without hemorrhage. (30)

GENETICS:

Familial cancer syndromes:

Though nearly 95% of medulloblastomas are sporadic in origin, initial genetic assessment was obtained by studying the familial cases of medulloblastoma. Familial syndromes

associated with medulloblastomas are Gorlin syndrome(17), Turcot syndrome(31) and Li-Fraumeni syndrome.(32)

Gorlin syndrome (also known as Naevoid basal cell carcinoma syndrome) occurs due to a mutation in the tumour suppressor gene patched 1(*PTCH1)* which codes for a membrane bound receptor involved in developmental sonic hedgehog pathway. Patients with Gorlin syndrome are 600 times more prone to develop medulloblastoma. Almost 10% of the sporadic cases display *PTCH 1* mutation.(33)

Turcot syndrome is characterised by colonic polyps, colorectal cancers and neuro-epithelial tumours. Two types have been described. Type 1, known as Hereditary non polyposis colorectal cancer syndrome, occurs as result of DNA mismatch repair and is associated with early onset malignant glioma.(34) Type 2 occurs as a result of germline mutation of *TSG APC* gene and is associated with an increased incidence of central nervous system tumours. Other familial syndromes associated with medulloblastomas are Fanconi's anaemia, Rubenstein–Taybi syndrome (35) and Coffin-Siris syndrome.(36)(37)

Oncogene amplification in medulloblastoma**:**

The amplification of oncogenes is a well-defined mechanism for disease progression in medulloblastoma. *MYC* oncogene is responsible for transformation, cell proliferation, differentiation and genomic instability. *MYC amplification* is observed in approximately

4% of medulloblastomas.(38) Increased expression of N *MYC* promotes proliferation of the cerebellar granular cells with anaplastic histology.(39) Over expression of N *MYC* is associated with metastatic medulloblastoma.(39)

MYC L amplification is rarely observed (1-2%) in medulloblastoma.(40) MYC proteins are regulated by their formation of hetero-complexes with *MYC* associated factor X(MAX), which promotes transcription of the genes containing E-box binding site.(41) MAX is also able to form repressive complexes with MAD family of proteins, which govern the transcriptional status of the targets of the MYC protein. MYC and MYC N amplification occurs rarely(42) in adult medulloblastomas and if present, has a poor outcome.(20)

Recurrent chromosomal abnormalities:

The most commonly observed chromosomal defect in medulloblastomas are the abnormalities of chromosome 17. Loss of chromosome 17p is seen in 30-40% of cases and is commonly associated with reciprocal gain of 17q. This is known as isochromosome 17q. (40) (43) 15% of tumours have gain of 17q without loss of 17p whereas gain of whole chromosome 17 is observed in 5-10% of tumours. Loss of chromosome 17p in isolation is observed in 20% of the cases and is also seen to be associated with a poorer prognosis.(44)

OTX2 in medulloblastomas(45):

OTX 2(orthodonticle) is a transcription factor which is essential for the growth and development of the brain. During the development of the brain OTX2 is expressed in

progenitor cells in prenatal period. This transcription factor is found to be expressed in areas of the forebrain, external granular layer and the developing internal granular layer of the cerebellum. In paediatric brain tumours OTX2 transcription factor is expressed at high levels. OTX2 transcription factor has functional interaction with the MYC oncogenes which results in high level expression of these both transcriptional factors in medulloblastomas. In medulloblastomas high level expression of OTX2 transcription factor results in cell proliferation. Loss of OTX2 expression has also been found responsible for myogenic differention in medulloblastoma.(46)

Abnormal activation of embryonal signaling pathways:

Cell signaling pathways play an important role in the developmental processes. Inappropriate activation leads to inappropriate growth and differentiation. WNT and SHH pathway have been implicated in the pathogenesis of medulloblastoma.(47)

Sonic hedge hog (SHH) pathway:

SHH pathway is an important regulator of the embryonic development. It plays a vital role in stem cell maintenance, cell differentiation, proliferation and tissue polarity. In 1980, hedgehog gene spiked phenotype was observed in mutant *Drosophila* larvae.(48) There are 3 mammalian homologues of *Drosophila* hedgehog gene namely Sonic, Indian and Desert hedgehog. SHH pathway has diverse effects in different cellular contents. Sonic hedgehog acts as a morphogen in cell fate differentiation and mitogen in development of organs.(49) SHH gene plays a crucial role in cerebellar development.

In adults it plays a vital role in proliferation of the adult neural stem cells.(50) Other functions include tissue repair, renewal and tissue homeostasis.

During normal SHH pathway signaling, SHH ligand binds to PTCH 1, which releases the receptor smoothened (SMO) from inhibition which in turn activates the signaling cascade. This event is possibly mediated by G-proteins, that activates the GLI family of transcription factors, by forming an activated complex, GLI A that transcriptionally activates SHH pathway target genes such as cyclin D1 and MYC N.(50) In absence of SHH ligand, GLI proteins are proteolytically processed to generate the repressive complex, which prevents SHH target genes from being transcribed.(51) GLI 1 and GLI 2 are found to be transcriptional activators and GLI 3 is found to be a transcriptional repressor.(52) In the absence of SHH ligand repressor, GLI R is produced.

Cerebellar development and role of SHH pathway:

Developmental processes that occur within the cerebellum are related to the origin and pathogenesis of medulloblastoma. The oncogenic abnormalities in the signaling pathways may result in the formation of different types of medulloblastoma.(44)

During early stages of cerebellar development, cells are derived from two distinct germinal zones; one from precursor cells in the roof of the fourth ventricle, which gives rise to GABAnergic precursors, including Purkinje cells; the other from cells within the rhombic lip, which comprises of GNP cells, which produce the external granular layer (EGL).(44) The EGL persists into the second year up to the post-natal life.

The SHH pathway plays a vital role in the development of GNP cells and also controls the expansion, differentiation and migration of GNPs in the EGL of the cerebellum.(53) The importance of the SHH pathway was further studied by inhibition of SHH signaling pathways in mouse models of cerebellar development. This was found to result in a marked decrease in the proliferation of the EGL(54) conversely, recombinant SHH drives the proliferation of GNPs.(55)

Activation of the SHH signaling pathway characterizes approximately 25% of medulloblastomas. Mutations in SHH pathway components (*PTCH1*, *SUFU*, SMO) have also been described for subsets of medulloblastomas(56). The mechanisms by which the SHH pathway can drive medulloblastoma have been elucidated in mouse models of the disease. 10-15% of *PTCH1* +/- mice go on to develop medulloblastoma(57) and express high levels of *GLI 1*, consistent with activation of the SHH pathway. More than half show residual populations of GNP cells at the surface of the cerebellum that fail to undergo terminal differentiation and migration to the internal granular layer, suggestive that medulloblastoma arises from these residual populations in this model.(57)

Activation of the SHH pathway is observed in the majority of infant cases (42) and is also associated (although not exclusively) with a desmoplastic phenotype. A similar enrichment has been observed in adult cases, where an incidence of 50% has been reported. (43) Multiple studies have identified distinct sets of differentially expressed genes that characterize medulloblastoma with an activated SHH signaling pathway (44)

(45) (46). No difference in survival has been reported between adult and childhood SHH medulloblastomas. (43)

WNT pathway:

The WNT signaling pathway plays an important role in embryogenesis and cancer and is also important in the regulation of normal physiological processes in adults. It was found that it is recognized to be important in developmental biology, and its relevance to cancer became evident when the interaction with the tumour suppressor genes APC and β-catenin was identified.(58) The identification of mutations in the *APC* gene, in families affected with Turcot syndrome linked their syndrome to inappropriate activation of the WNT signaling pathway. After this it was identified that up to 85% of sporadic colorectal cancers were associated with truncating mutations in *APC.*(59) Gain of function mutations in *CTNNB1* (the β-catenin gene) which is most commonly observed in WNT pathway activated medulloblastomas however, have rarely been reported in colorectal cancers(60).

In the absence of WNT ligands, cytoplasmic β-catenin is recruited into a destruction complex, consisting of APC, Axin and Glycogen synthase kinase-3β (GSK-3β), where its N-terminal is phosphorylated by casein kinase-1α and GSK-3β.(60) After phosphorylation, β-catenin is targeted for proteosome mediated degradation, ensuring that cytoplasmic levels of β-catenin remain low. (60)

Activation of the canonical WNT signaling pathway is initiated by secreted WNT proteins binding to Frizzled receptors in the plasma membrane. This interaction can be

inhibited by several proteins, including the secreted frizzled related proteins (SFRPs), Dickkopfs (DKKs) and WNT-inhibitory factor 1 (*WIF1*).(60) In further steps, Disheveled (*DSH*) is recruited to the plasma membrane, where it interacts with Frizzled to mediate the translocation of Axin to the plasma membrane and inactivation of the destruction complex. This inactivation enables the cytoplasmic stabilization and subsequent translocation of β-catenin to the nucleus.

In the nucleus, β-catenin forms a transcriptionally active complex with the lymphoid enhancer factor (LEF) and T cell factor (TCF) transcription factors. Target genes of the transcriptional complex include the proto-oncogene, *MYC*, proliferative genes such as cyclin *D1* and cell signaling genes (*VEGF, FGF4* and *FGF18*).

Cerebellar development and WNT pathway:

The role for the WNT pathway in cerebellar development is poorly understood, although it has been shown that deletion of the *WNT-1* gene, blocks cerebellar development by preventing the formation of the midbrain-hindbrain junction from which the cerebellum is developed. (61) Recent work in mouse models reported the expression of WNT pathway target genes in the lower rhombic lip of the cerebellum at day 11.5 of embryonic development, and the dorsal brainstem at day 15.5 (62)(19) , which helped to identify a cell of origin for medulloblastomas with an activated WNT signaling pathway.

WNT pathway in medulloblastoma:

The most commonly observed mutations in medulloblastoma with an activated WNT signaling pathway are activating mutations that affect the phosphorylation domain of the

β–catenin protein, affecting 8% of sporadic medulloblastomas and~95% of WNT-activated medulloblastomas.(63) Now, in the absence of WNT ligand, the β-catenin protein can no longer be phosphorylated by the complex of APC, Axin and GSK-3β. Instead, unmodified β–catenin is free to translocate to the nucleus and initiate transcription of proliferative WNT pathway target genes. Mutations in other WNT pathway components have also been identified. Individuals affected by Turcot syndrome harbor mutations in *APC* (64) , one of the proteins responsible for targeting β-catenin for degradation and rare mutations of AXIN have also been reported.(50) The majority of the WNT pathway activated tumours are of classic histology(65), occurs in non infants and have dual peak of incidence at 10 and 20 years. They have also been associated with a favourable prognosis.(66) WNT activated tumours have distinct molecular and genomic defects that enable classification of these tumours.

NOTCH signaling pathways in medulloblastomas:(67)

Medulloblastomas can arise from various deregulated pathways including NOTCH signaling pathway. NOTCH pathway plays a vital role in the normal physiological cerebellar development during embryogenesis. It plays an important role in cell differentiation, proliferation and apoptosis. Activation of NOTCH signaling pathway requires the interaction ligands (JAG1, JAG2, DLL1, DLL3 and DLL4) and receptors (NOTCH 1-4).

As ligand binding proceeds, it triggers the proteolytic cleavage of NOTCH receptors. Once released into the cytoplasm, the NOTCH intracellular domain translocates into the nucleus and activates a series of transcriptional regulatory pathways. The aberrancies were noted in four ligands - JAG1, *JAG2*, *DLL1*, and *DLL4*. It was found that there is over expression of JAG1, DLL1 and down regulation of JAG2 and DLL4 in medulloblastomas. (67)

Subpopulation of Group 3 cases expressing high *JAG2* levels and up-regulation of NOTCH ligand *DLL1* in Group 4 cases and a down-regulation of *DLL3* in Groups 3 and 4 tumors has been observed . It was found that association between the MYC and JAG2 ligands plays a crucial role in group 3 cases. High levels of expression of JAG2 correlate with large cell/anaplastic morphology and high rates of metastasis.(67)

PATHOLOGY:

Medulloblastoma were originally described as small blue round cell tumours on light microscopy.(3)

HISTOLOGY

Medulloblastoma is composed of closely packed cells with round-to-oval shaped hyperchromatic nuclei surrounded by scanty cytoplasm.(26) Neuroblastic (Homer Wright) rosettes are often associated with medulloblastomas.(3) Marked nuclear pleomorphism and atypia leads to anaplasia in medulloblastomas. Vascular hyperplasia and areas of necrosis are quite uncommon, but when present necrosis can show a pseudopalisading pattern. The most common type of differentiation in medulloblastoma

is neuronal which on immunostaining shows positivity for synaptophysin.(13) The presence of glial differentiation with astrocytic phenotype is rare in medulloblastomas. (13)

According to the 2007 WHO classification, the Histological subtypes include:

1. Classic variant
2. Desmoplastic variant
3. Medulloblastoma with extensive nodularity (MBEN)
4. Anaplastic variant
5. Large cell variant

1. Classic variant:

The classic variant constitutes about 70-80% of all medulloblastoma cases. (29)

This variant is hypercellular and composed of cells with medium sized hyperchromatic nuclei surrounded by scant cytoplasm. .Nucleoli are not prominent.Nuclear moulding may be seen due to high cell density. Mitotic activity is brisk as apoptosis. This variant is more common in children. Most of these tumours are seen in a midline location. Neuroblastic rosettes (Homer Wright rosettes) representing neuronal differentiation are found in about 40% of medulloblastoma and a characteristic feature of this variant.

Necrosis and vascular endothelial proliferation are not common.(68) Linear arrays (streaming) is occasionally seen.

2.Desmoplastic/nodular variant:

This variant constitutes about 10-15% of all medulloblastoma cases with a higher prevalence among infants and adults.(7) It is more frequently seen in the cerebellar hemisphere than in the fourth ventricle.(63) Desmoplastic variant is characterized by nodular reticulin free zones (pale islands) surrounded by densely packed highly proliferative cells. Internodular areas are more densely packed and pleomorphic as compared to the nodular areas that are pale in comparsion due to increased amount of cytoplasm. Nucleoli are not prominent. Nuclear moulding may be seen due to high cell density. Mitotic activity is brisk as apoptosis. Desmoplasia was previously associated with improved prognosis in infants. Its prognostic value in non-infants is unclear. Extent and increased density of nodules have improved prognosis.(69).

3. Medulloblastoma with extensive nodularity:

This variant which was previously described as cerebellar neuroblastoma, comprises about 1-2% of all medulloblastomas.(70) It has an expanded lobular architecture because of the unusually large reticulin free zones which are rich in neuropil like tissue. This variant has round cells with uniform nuclei and high level of neuronal differentiation and low proliferation index.(71) It is seen nearly exclusively in infants and is associated with a favourable prognosis. (72)

4. Anaplastic variant:

Approximately 10-20% of medulloblastomas comes under this variant. Presence of increased nuclear pleomorphism, nuclear moulding, cell-cell wrapping and high mitotic activity characterise this variant of medulloblastoma. Apoptosis is prominent in this

variant.(73) Presence of aforementioned features in focal areas is not sufficient for diagnosis of anaplastic variant.(3)

5. Large cell medulloblastoma:

Approximately 2-4% of medulloblastoma comes under the large cell variant. (73) Monomorphous population of cells with large, round, vesicular nuclei, prominent nucleoli and variable amount of cytoplasm are noted. Mitotic and apoptotic figures are predominant. Areas of large cell can be seen alongside the more typical histological subtype, classic, desmoplastic, nodular and medulloblastoma with myogenic differentiation. This variant is characterised by poor prognosis.

Medulloblastoma with myogenic or melanotic differentiation:(74)

Previously described as medullomyoblastoma , these are tumours that show focal skeletal muscle differentiation.(74) These could be in the form of rhabdomyoblast or mature skeletal muscle. The cells are positive for desmin, myoglobin and myosin, but not smooth muscle actin. (75) Genetic changes in medulloblastoma with myogenic differentiation are similar to other subtypes of medulloblastomas.

Medulloblastomas with melanocytic differentiation were previously known as melanotic medulloblastoma.(76) These are tumours that show melanocytic or retinal pigmented epithelial differerentiation. The melanotic cells can occur in different variants of medulloblastoma. The tumours with myogenic or melanotic differentiation are now considered as morphological patterns and not as variants of medulloblastoma. (76)

Rarely encounters medulloblastoma with chondroid differentiation or osseous or epithelial differentiation.(77)

The identification of the different histological variants is clinically useful as the tumors with desmoplastic histology are associated with better outcomes while large cell/anaplastic medulloblastomas have a poorer prognosis.

The evolution of classification from histology to molecular:

Current management of medulloblastomas focuses on a maximal safe resection followed by craniospinal radiotherapy and adjuvant systemic chemotherapy. When patients are stratified into two groups: average risk including those children > 3 years of age who have undergone a gross total resection and no evidence of leptomeningeal metastases (M0) and high risk being those patients ≤3 years, residual tumor >1.5cm^2 or with evidence of leptomeningeal metastases outcomes are clearly distinct. Average risk patients enjoy a 5-year survival of about 85% while the survival drops to 60-65% in the high risk group. (78)

With the recognition that signaling pathway abnormalities, specifically SHH and Wnt, play a role in the development and progression of medulloblastomas, it also became apparent that this disease is more heterogeneous than previously thought. Abnormalities within each signaling pathway seemed to result in distinct phenotypic presentations that had varying biological behavior and aggressiveness leading to different clinical

outcomes. (79) Thus the traditional method of stratifying patients purely on a clinical and radiological basis is bound to be fallacious since they do not incorporate this new molecular information. If medulloblastomas are sub-grouped on a molecular basis and this information integrated with the clinicoradiological information it is likely that the risk stratification and prediction of outcomes will improve. The obvious benefit of improved risk stratification for children with medulloblastomas is the reservation of highly toxic chemotherapy for the high risk group and the adoption of a more moderate approach for the low risk group thus optimizing outcomes versus treatment complications. (80)

Evolution of subgrouping in Medulloblastomas:

It is evident from the differences between the WHO 2000 and 2007 classification of CNS tumors that medulloblastomas were recognized to be distinct subgroup of primitive neurectodermal tumors. Their morphological similarities with PNETs with their small blue round cell appearance was misleading because it was soon demonstrated by Pomeroy et al (81) using gene expression profiling that medulloblastomas were genetically distinct from other malignant embroyonal brain tumours. Amongst neuropathologists it was also apparent for many years that the heterogeneous morphological appearance of medulloblastomas lends itself to be classified broadly into those that are desmoplastic (medulloblastoma with extensive nodularity and nodular desmoplastic medulloblastomas) and those that are large cell or anaplastic. (82)

The histological segregation into these two groups led to the recognition that their outcomes were different with poorer outcomes for the latter group. Thus the finding that mutations in the PTCH1 gene coding for PTCH1, a receptor for SHH, and two downstream targets of SHH namely GLI and N-Myc were highly correlated with the desmoplastic variant of medulloblastomas implicated SHH dysregulation in the pathogenesis of one subset of medulloblastomas.

Later, Gibson et al(27) confirmed that different subtypes of medulloblastoma have distinct developmental and cellular origins. The lower rhombic lip and dorsal brain stem expressed the WNT subtype signature and consistent with this is the finding that tumors with the WNT signature arise in the floor of the fourth ventricle and infiltrate the brain stem. In contrast the gene expression of the SHH subtype was seen in the upper rhombic lip and cerebellar hemisphere and SHH pathway tumors are frequently seen to arise in the cerebellar hemispheres.

Between 2006 and 2011, researchers in separate laboratories across the world began intensive work on further characterizing molecular subgroups in medulloblastomas(20) (83)Using different but complementary techniques ranging from gene expression profiling, mRNA expression profiles, mRNA transcriptome profiling and high density SNP array and miRNA analysis these authors arrived at 5 distinct molecular subtypes, A to E.(83) They seemed to concur on the findings that each subtype had specific gene signatures and distinctive genetic abnormalities but more importantly that each subtype had specific clinicopathological features. The subtypes were characterized by Wnt

signaling (Group A), SHH signaling (Group B), neuronal differentiation gene expression (Groups C and D) or photoreceptor gene expression (Groups D and E).

Furthermore it was determined that tumors with a C-MYC copy number gain were in a subgroup with a poor prognosis.(84)(46)The work from Northcott et al(12) in 2011 using integrated genome-wide DNS copy number and mRNA expression profiles crystallized the classification system into the current recommended grouping.

In addition, using microarray data they developed antibodies against the subgroup-specific signature genes enabling a immunohistochemistry-based classification thus obviating the need for fresh frozen tumor tissue for genomic analysis that was expensive and not freely available. Thus IHC for DKK1 indicating the WNT pathway; SFRP1 indicating the SHH pathway, NPR3 for Group C and KCNA1 for Group D provided a simple method for subgrouping medulloblastomas.

From India, the Tata Memorial Cancer Center provided microRNA data that seem to be consistent in classifying tumors into the WNT pathway subgroup and SHH subgroup based on over and under expression of certain microRNAs respectively.(16)

In 2012 a consensus meeting brought together current knowledge on molecular signatures of medulloblastomas and recommended 4 subgroups namely: WNT, SHH, Group 3 and Group 4.(85) The first two named the signaling pathways were considered to be instrumental in the pathogenesis of these tumors while the last two were merely called

Group 3 and 4 because until that time no specific pathways were implicated in their pathogenesis.(85)

Relevant features of molecular subgroups:

These molecular subgroups can predict the patient's survival with more accuracy than clinical risk stratification. (53)

WNT pathway medulloblastomas:

This subgroup is rare constituting only 10% of all medulloblastomas, yet they have the best prognosis with 5-year survivals of more than 90%. They are seen predominantly in the age group of 10-12 years and are less common in less than 4 years of age and are very rare in infants. . It is equally distributed in both genders.(13) Histologically this group is characterized by the classic type of medulloblastoma and some have large cell/anaplastic morphology. (53)

The WNT signaling pathway plays a role in regulating embryogenesis of the brain and one of the genes in this pathway, the adenomatous polyposis coli gene APC is mutated in Turcot's syndrome that predisposes patients to colon cancer, glioblastoma and medulloblastoma.(86) More than 90% of WNT pathway medulloblastomas have mutations in CTNNB1, a gene coding for β-catenin that interacts with other transcription factors to activate the WNT signaling pathway.(7) Mutant β-catenin protein accumulates in the nucleus of tumor cells. In addition WNT medulloblastomas also show loss of one copy of chromosome 6.(7) Taken together, IHC positivity for β-catenin and monosomy 6 is diagnostic for WNT pathway medulloblastomas.(12) Other mutations found in this

pathway include AXIN 1 and AXIN 2. This subgroup is also associated with amplification of MYC genes and infrequent metastasis.(12)

SHH pathway medulloblastomas:

This subgroup is more frequent than WNT tumors occurring in approximately 30% of all medulloblastomas.(10) This group is common both in infants and in patients > 16 years of age.(14) Many SHH tumors display desmoplastic histology, in fact the MBEN variety is almost exclusively seen in this subgroup alone. The majority are lateralized to the cerebellar hemispheres consistent with their proposed origin from the granule neuron precursor cell.(87)

The SHH gene codes for the protein patched homologue 1 (PTCH1) that is a receptor for SHH and other hedgehog homologues. SHH signalling drives normal cerebellar development, however, unrestrained SHH activity can lead to medulloblastoma tumorgenesis.(88) The fact that PTCH1 mutations occur in only 25-30% of SHH-associated medulloblastomas indicates that this subgroup is quite heterogenous and caused by other genetic abnormalities including SMO and SUFU mutations and amplifications of SHH, GLI2 and N-MYC have been noted.(89) Most infants carry the PTCH1 or SUFU mutations while PTCH1 and SMO mutations typically occurs in adults.(14) Immunohistochemistry with SFRP1, GLI and GAB-1 helps in diagnosing this subgroup.(72) They are associated with intermediate prognosis.(7)

Group 3 Medulloblastomas:

This subgroup carries the worst prognosis with a 5-year survival around 50%. (13) Fortunately they occur in only 1/4th of all patients, boys being more frequently involved, and they almost never occur in adults.(87) The desmoplastic histology is almost never seen in this subgroup; instead the LCA variety predominates occurring in about 40% of patients.(87) In keeping with their poor prognosis, they tend to metastasize via the CSF spaces at presentation.(90) It appears that copy number gains of 17q and C-MYC or N-MYC amplification impart the poor prognosis to patients in this group(87). Nonetheless, while more than 50% of Group 3 tumors do not display any specific genetic abnormalities, extensive variations in chromosome structure exist, the significance of which are poorly understood as far as tumorigenesis is concerned.(87) Group 3 is further classified into 3 alpha and 3 beta.(90) OTX2 transcriptional factor is found to be associated with this subgroup. Other abnormalities include gain of chromosome 1q, loss of chromosome 5q and loss of chromosome 10q and has a poor prognosis.(22) .

Group 4 Medulloblastomas:

This subgroup is quite common comprising 35% of all medulloblastomas(85). There is a marked male predominance and these tumors are seen across all age groups, but less common in infants.(87) The prognosis in this group of tumors is between that of the WNT group and Group 3. Classic and Large cell histology are the common subtypes seen in Group 4. Chromosome 17 abnormalities are a hallmark of this subtype.(73) One of the common aberrations seen is isochromosome 17q.(7) HDAC 5gene locus is

located on the chromosome 17q, which is commonly amplified in this subgroups.(90) Other cytogenetic abnormalities include CDK6 and MYC N amplification.(7).

There are no familial syndromes associated with this tumor subtype, nor copy number alterations or genetic mutations frequent. (72) 35-40% of cases have metastatic disease at the time of first presentation(87). The overall 5-year survival ranges from >85% to 50%.(85)

Use of immunohistochemical markers in molecular sub classification:

The immunohistochemical(IHC) markers used are based on previously identified genetic signatures using Fluorescent insitu hybridisation, MicroRNAs, comparative genomic hybridisation and real-time PCR uniqueness.(12) IHC markers were used for molecular subclassification of medulloblastomas namely DKK1, SFRP1, NPR-3 and KCNA 1 based on the results of genome wide DNA copy number and mRNA expression profiles and it was found that these markers reliably classified medulloblastomas into four subclasses, DKK1 (WNT), SFRP1 (SHH), NPR3 (group C) and KCNA1 (group D)(7). However, both NPR-3 and KCNA 1 were subsequently found to be neither specific nor sensitive markers and lacked specificity as their expression was identified in all subgroups.(7)

Another study by Min et al (78), molecular subgrouping was based on nuclear expression of β-catenin for WNT subgroup, GAB-1 expression for SHH subgroup, NPR3 expression in group 3 and KCNA-1 expression in group 4 subroup of medulloblastomas

According to Ellison et al (10), four immunohistochemical markers to aid in molecular stratification. B-catenin was very useful in identifying tumours with WNT signaling pathway defects with almost 98% specificity. Cytoplasmic positivity for GAB-1 was seen in internodular areas of desmoplastic variants and therefore useful in identifying tumours with defect in SHH pathways. FLI A and YAP-1 negative expression was reported to be markers of non-WNT/SHH pathway. The immunohistochemistry profile in classifying the molecular subgroups is mentioned in the table below.

Table 3 : Immunoprofile of molecular subgroups of medulloblastoma. (79)

Molecular Group	Immunoreactivity			
	GAB1	β - catenin	Fillamin A	YAP1
SHH	Cytoplasmic	Cytoplasmic	Cytoplasmic	Nuclear + Cytoplasmic
WNT	Negative	Nuclear + Cytoplasmic	Cytoplasmic	Nuclear + Cytoplasmic
Non-SHH/WNT	Negative	Cytoplasmic	Negative	Negative

MicroRNAs in molecular subclassification of medulloblastomas:(16)

MicroRNAs (miRNAs) are RNA molecules with 18 to 22 long nucleotide sequences, which regulate expression of the protein coding genes.(16) These miRNAs bind to multiple target genes at their complementary sequences in the 3′untranslated regions,

resulting in gene silencing. These micro RNAs target several genes and its alteration has been reported in several malignancies. Deregulation in micro RNAs plays a vital role in pathogenesis of various malignancies. Micro RNAexpression profile has been found to have diagnostic and prognostic potential in the classification of various cancers.(16)

In a study by Kunder et al(16), MiR-182 was overexpressed in all WNT medulloblastomas and in many Group 3 and some Group 4 medulloblastomas, while miR-204 was overexpressed in all WNT medulloblastomas and in most Group 4 medulloblastomas. MiR-182, miR-135b and miR-204 were found to be under expressed in SHH medulloblastomas. MiR-135b was found to be overexpressed in Group 3 and Group 4 tumors. MiR-592, a miRNA located within the GRM8 gene, was overexpressed in Group 4 medulloblastomas. MiR-10b was expressed at the highest level in WNT medulloblastomas, followed by Group 3 medulloblastomas. MiR-376 expression was found to be significantly higher in Group 4 medulloblastomas compared with Group 3 medulloblastomas.

Molecular Subgrouping using protein coding gene:(16)

The 12 protein-coding genes and 11 microRNAs help in classification of four molecular subgroups of medulloblastomas. Classification of medulloblastomas into the 4 molecular subgroups was demonstrated using a set of 12 protein-coding genes and 9 miRNAs as markers by a real-time RT-PCR based assay with an overall accuracy of 97%. Molecular classification with the help of 9 miRNAs have accuracies of 100% for WNT subgroup, 93.3% for SHH pathway, 85.7% for group 3 and 100% for Group 4.

Group 3 and Group 4 tumors have distinct survival rates but with overlapping gene expression. MiR-182 and miR-592 was found to be overexpressed in Group 3 tumors and group 4 tumours respectively. MiR-592 and miR-182 are found to be markers for Group 3/Group 4 classification and also markers for risk stratification of non-WNT, non-SHH medulloblastomas.

Risk stratification:

Histology, molecular features and genetic profiling have refined the stratification of disease risk in medulloblastoma.(91) Various factors that help in disease stratification are age, completeness of resection, histological subtypes and genetic markers(92).

Modified Chang system is still in use for disease risk stratification(92). Based on the histology, metastasis and genetic profiling, risk stratification can be predicted as low risk, standard risk and high risk.(93) Clinical risk stratification includes only standard risk and high risk tumour.

Current therapy includes surgical resection, craniospinal irradiation and high dose chemotherapy based on the risk stratification.(78) Patients with leptomeningeal metastasis or incomplete resection are considered to be at high risk.(92)

Low risk medulloblastomas:

Nuclear positivity with β-catenin, absence of metastasis, large cell morphology/anaplastic morphology and MYC amplification are considered to be low risk.(94)

High risk medulloblastomas:

Medulloblastomas with metastatic disease, large cell/anaplastic morphology and MYC amplification are high risk group.(94)

Standard risk medulloblastomas:

Medulloblastomas which lack discriminating features like metastasis, MYC amplification and large /anaplastic morphology were found to have standard risk.(98)

Cytogenetic abnormalities also play a role in risk stratification. GLI 2 amplification, 14q loss and leptomeningeal dissemination identifies the high risk and standard risk patients. Presence of GLI 2 amplification alone is identified in patients with poor prognosis. GLI2 amplification, 14q FISH and metastatic status predicts prognosis for patients having SHH medulloblastomas.(13) Chromosome 8q loss and chromosome 1q gain are the only good prognostic markers.(94)

Treatment:

Treatments for standard risk patients:

Patients with standard risk and more than 3 years of age at the time of diagnosis are treated with protocols designed to reduce, as far as possible, neuro-cognitive and neuro-endocrine sequelae, thereby maintaining a low rate of relapse. Progression free survival rates for 5 years is 79%-81%.(78)

Treatment for high risk patients:

High-risk and non-infant patients are categorized by the presence of metastatic disease (M1 / M2 / M3) and /or residual disease. Five -year progression free survival rates for

high risk patients is approximately 60%.(29) The goal in this group of patients is to achieve a cure, rather than avoidance of neuro-cognitive and neuro-endocrine sequelae.

Clinical course:

Survivors of medulloblastoma will have long term sequelae due to damage from the tumour and from radiotherapy treatment. The extent of impairment is inversely related to the age of the patient.(95) The neuro-endocrine and spinal damage can affect growth, bone development and can lead to early puberty. Adverse effects of chemotherapeutic regimes include ototoxicity, infertility and can also lead to secondary malignancies. Survivors may have lifelong social and educational difficulties resulting in significant morbidity.(96)

MATERIALS AND METHODS:

All the procedures carried out in the present retrospective study were approved by the Institutional Review Board of Christian Medical College, Vellore. There were a total of 123 medulloblastomas retrieved from the database of the Department of General Pathology, Christian Medical College, Vellore from January 2004 to December 2014. Haematoxylin and eosin (H&E) stained and mounted slides and paraffin embedded tissue blocks were retrieved from the departmental archives.

The slides were reviewed for the assessment of the blocks and confirmation of diagnosis. One hundred and thirteen medulloblastoma samples had sufficient tissue in the paraffin block for performing additional immunohistochemical studies. Clinical details were obtained from the clinical workstation. A detailed review of the H&E stained slides and previous immunohistochemical studies was done as detailed in the proforma shown overleaf:

Title of the study: Histological and molecular subtyping of medulloblastoma using immunohistochemical markers.

Biopsy no: Name:

Hospital no: Age: Gender:

Histological features:

A) Pattern: 1) Nodular 2) Lobular 3) Sheets 4) Clusters 5) Streaming
B) Cellularity: 1) Hypocellular 2) Hypercellular
C) Size and shape: 1) Small round cells 2) Large cells
D) Homer Wright rosettes: 1) Present 2) Absent
E) Cytoplasm: 1) Scant 2) Variable amount of eosinophilic cytoplasm.
F) Nuclear features: 1. Nuclear pleomorphism 1) Mild 2) Moderate 3)Marked
2. Nuclear moulding 1) Present 2) Absent
3. Prominent nucleoli 1) Present 2) Absent
G) Marked cytological atypia 1) Present 2) Absent
H) Mitotis 1) High 2) Low
I) Apoptosis 1) Present 2) Absent
J) Necrosis 1) Present 2) Absent
K) Differentiation 1) Neuronal 2) Myogenic 3) Melanotic 4)Glial
L) Nodular reticulin free zones 1) Present 2) Absent
M) Histological subtype
1) Classic 2) Desmoplastic 3) MBEN 4) Large cell variant 5) Anaplastic

N) Immunohistochemistry:

IHC	**Cytoplasmic**	**Nuclear**	**Cytoplasmic+Nuclear**
Beta catenin			
GAB-1			
NPR-3			

Each reviewed case was classified into one of five histological subtypes as follows:

The tumour was sub classified into each of the variants based on the following features:

Classic variant:

Cases with closely packed round to oval cells with hyperchromatic (carrot shaped) nuclei surrounded by scant cytoplasm and high mitotic activity were classified as the Classic variant. Presence of Homer Wright rosettes was noted.

Desmoplastic/nodular variant:

Cases with nodular pattern and reticulin free zones (pale islands) surrounded by densely packed cells with a high proliferation rate and dense intercellular reticulum fiber network were classified as the Desmoplastic variant. Dense collagenous and reticulin fibres in the absence ofa nodular pattern was not considered to be a feature of the Desmoplastic or nodular variant.

Medulloblastoma with extensive nodularity:

Cases with expanded lobular architecture with enlarged reticulin free zones rich in neuropil like tissue were described as medulloblastoma with extensive nodularity.

Large cell medulloblastoma:

Cases with a monomorphous population of large cells with large, round, vesicular nuclei, prominent nucleoli and variable amount of cytoplasm with abundant mitotic and apoptotic figures were categorized as Large cell medulloblastomas. These cells lacked cohesiveness.

Anaplastic:

Cases with the presence of increased nuclear pleomorphism, nuclear moulding, cell-cell wrapping and high mitotic activity were described as the Anaplastic variant. Apoptosis was prominent in this variant. Presence of the above mentioned features in focal areas were not considered sufficient to diagnose the Anaplastic variant.

Myogenic differentiation:

Cases containing focal rhabdomyoblastic elements, as evidenced by a population of spindle shaped cells or large oval cells having abundant eosinophilic cytoplasm that were desmin positive, were considered as Medulloblastoma with Myogenic differentiation.

Slides of previously performed immunohistochemistry were reviewed, which included synaptophysin, neuron specific enolase (NSE) and glial fibrillary acidic protein (GFAP). On cases suspected to have myogenic differentiation we performed desmin and Myf4 immunohistochemistry in addition.

Immunohistochemistry for β-catenin, GAB-1 and NPR-3 were performed on all 113 cases using the Ventana Benchmark XT autostainer. Immunostaining for each antibody was standardized to obtain optimal dilutions. The β-catenin antibody was obtained from Sigma (purified mouse monoclonal immunoglobulin). Normal human colon was used as the positive control and 1:100 was found to be the optimal dilution. (Figure 1)

GAB-1 antibody was obtained from Santa Cruz (Rabbit polyclonal antibody) and human breast invasive ductal carcinoma, was used as the positive control with 1:50 as the optimal dilution. (Figure 2)

NPR3 was obtained from Sigma (Rabbit polyclonal antibody). For NPR3 normal human colon was used as the positive control and 1:100 was found to be the optimal dilution. (Figure 3)

The following procedure was followed for performing immunohistochemistry for these immunohistochemical markers.

Protocol for automated immunostaining:

1. Paraffin embedded tissue sections were cut at 3µ thickness and floated in poly L-lysine coated slides and incubated overnight at 37˚C.
2. These slides were then treated with 4% milk solution for 10 minutes to eliminate the hydrophobic effect and give a positive charge to the slides.
3. The slide labels were bar coded and loaded into the fully automated Ventana Benchmark XT autostainer. Individual protocols were designed incorporating the optimal dilutions for each antibody in the software attached to the machine. The steps included in this protocol were as follows:
    a. Deparaffinization
    b. Liquid coverslip application.

c. Heat induced antigen retrieval by treating with standard CC1 solution (pH patent with the company) for one hour at 90˚C.
d. Then the primary antibody was added and incubated for 40 minutes at 37˚C.
e. The secondary antibody (Multimer) was then added and incubated for 8 minutes.
f. Finally the slides were counterstained with Haematoxylin and incubated for 8 minutes, followed by incubation with the bluing reagent for 4 minutes.

At the end of each step from antigen retrieval to counterstaining with Haematoxylin, the slides were washed with reaction buffer.

The sections were dehydrated twice using 80% alcohol to remove the liquid coverslip. The slides were dried and the sections mounted in DPX. The antibody clone, dilution, source and technique used were as detailed in the table below:

Table 4 : Details of antibody clone, dilution and source

ANTIBODY	CLONE	DILUTION	SOURCE
β-catenin	pThr41	1:100	Purified mouse monoclonal immunoglobulin (Sigma)
GAB-1	H-198	1:50	Rabbit polyclonal antibody (Santa Cruz)
NPR-3	PrEST	1:100	Rabbit polyclonal antibody (Sigma)

Immunostaining for β-catenin was considered positive if there was uniform intense nuclear and/or cytoplasmic staining in more than 10% of tumour cells. Cytoplasmic staining in the absence of nuclear staining was considered negative. GAB-1 was considered positive if uniform intense cytoplasmic or cytoplasmic membrane labelling was seen in more than 10% of the tumour cells. NPR-3 was considered positive if

uniform intense cytoplasmic or cytoplasmic membrane labelling was seen in more than 10% of tumour cells.

Statistical Analysis:

Statistical analysis was done with the Chi square tests using SPSS software. A p value of less than 0.05 was considered significant. The patient demographic details and the morphological features of each tumor subtype were analyzed. A chi square test for correlation between morphological subtypes and immunohistochemical markers was performed. The specificity and sensitivity of β-catenin among classical and non-classical histological subtypes and GAB-1 among the desmoplastic and non-desmoplastic histological subtypes were calculated.

RESULTS

There were a total of 123 cases of medulloblastoma from January 2004 to December 2014. Of these 113 cases were included in the present study based on the defined inclusion and exclusion criteria that are outlined in the materials and method section.

The study population included 26 adults (23%) and 87 children (77%)(Figure 4). The mean age at diagnosis was 13 years(median 11years, range 0-38 years). The mean age amongst children was 9 years (median 9 years, range 0-18 years) while in adults it was 26 years (median 25 years, range 19-38 years).(Figures 5 and 6)

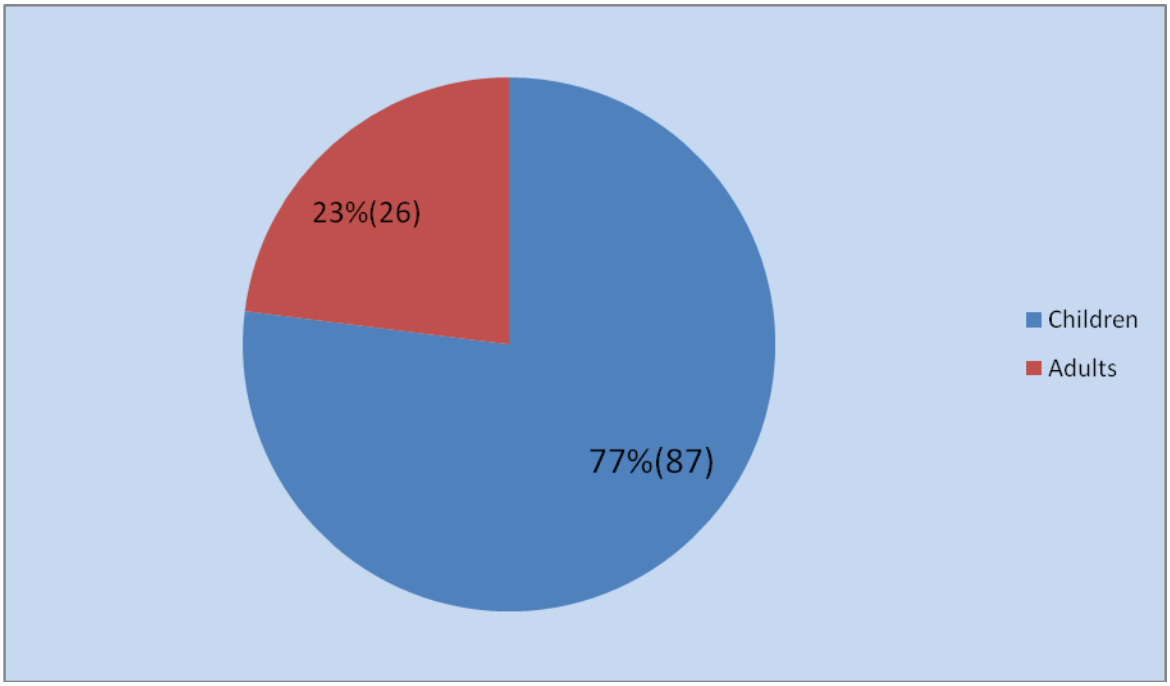


Figure 4:Age distribution among children and adults of medulloblastoma.

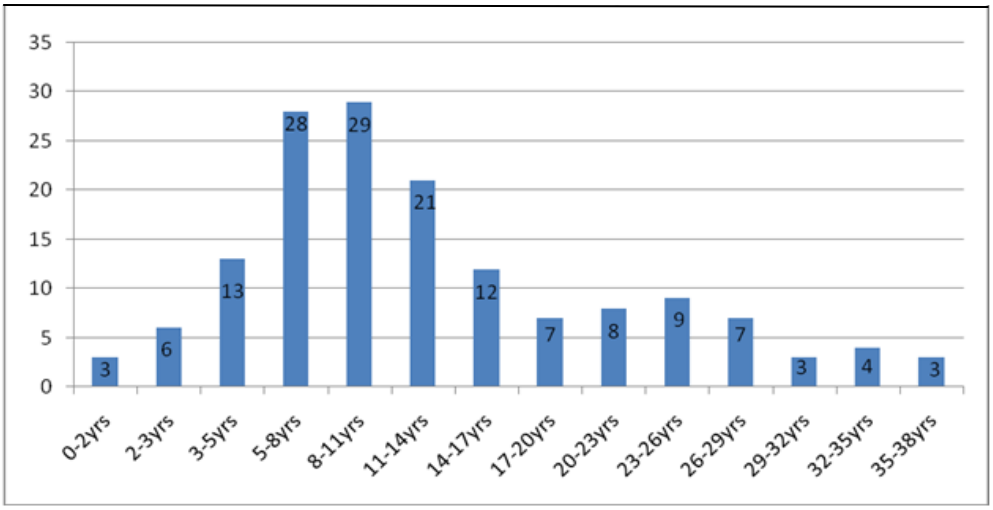


Figure 5: Age distribution of medulloblastoma

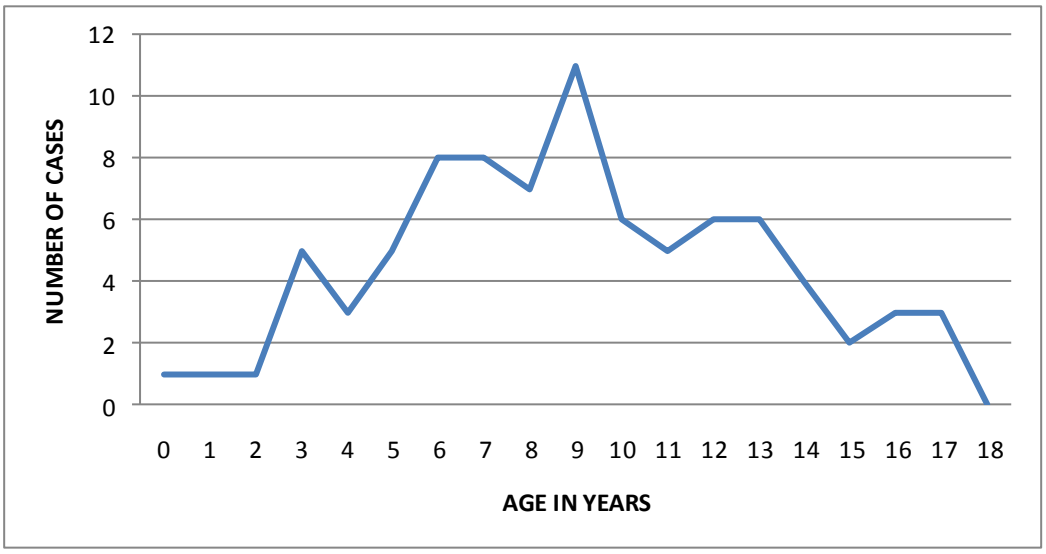


Figure 6:Age distribution of paediatric medulloblastoma

The M:F ratio was 2:1 with 66.4% (75 cases) males and 33.6% (38 cases) females (Figure 7). In children there was clear male preponderance with nearly two third of the cases being males (Figure 8), whilst in adults there was a near equal gender distribution (Figure 9).

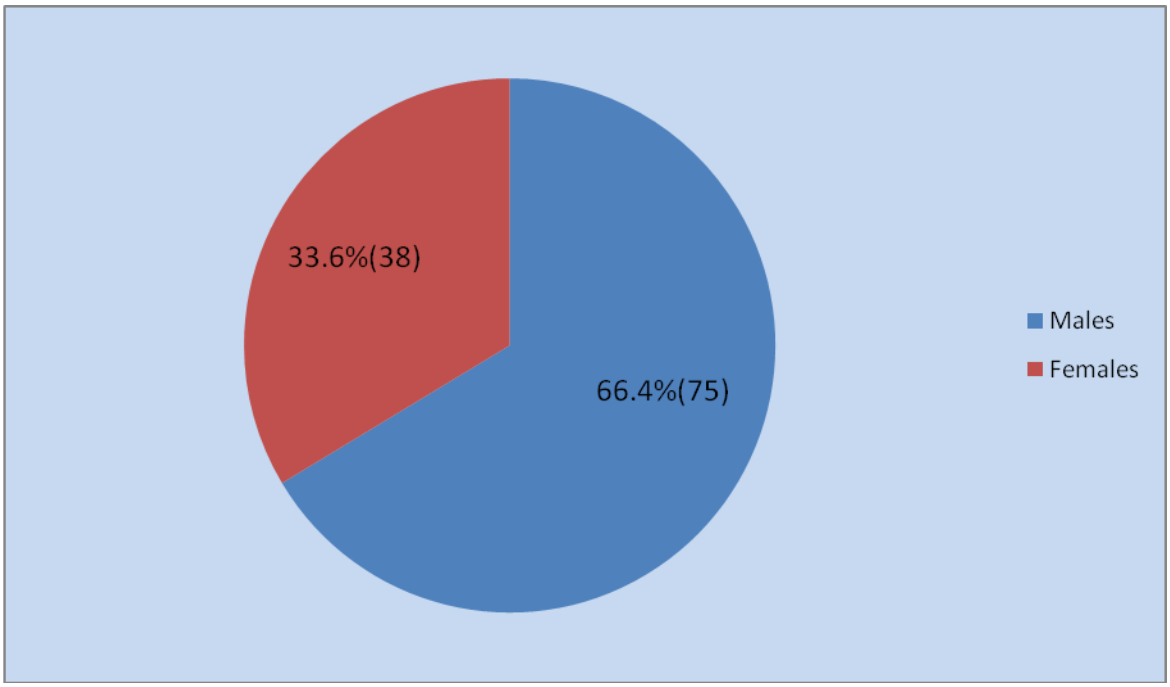


Figure 7:Gender distribution in medulloblastoma.

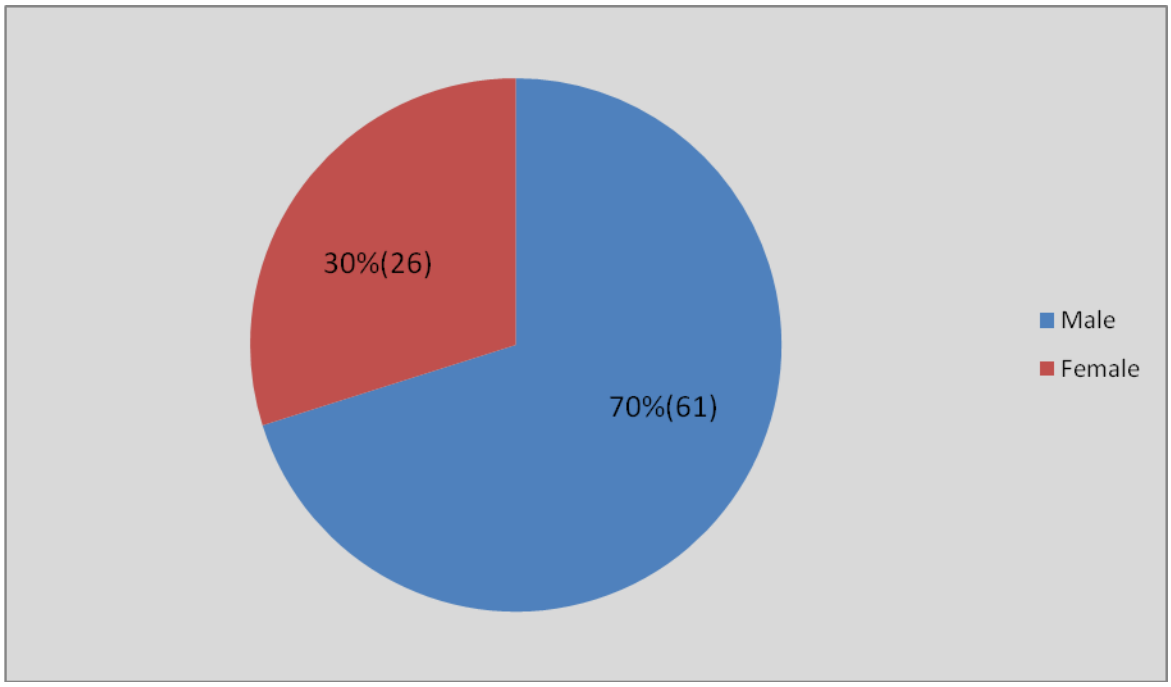


Figure 8:Gender distribution among children.

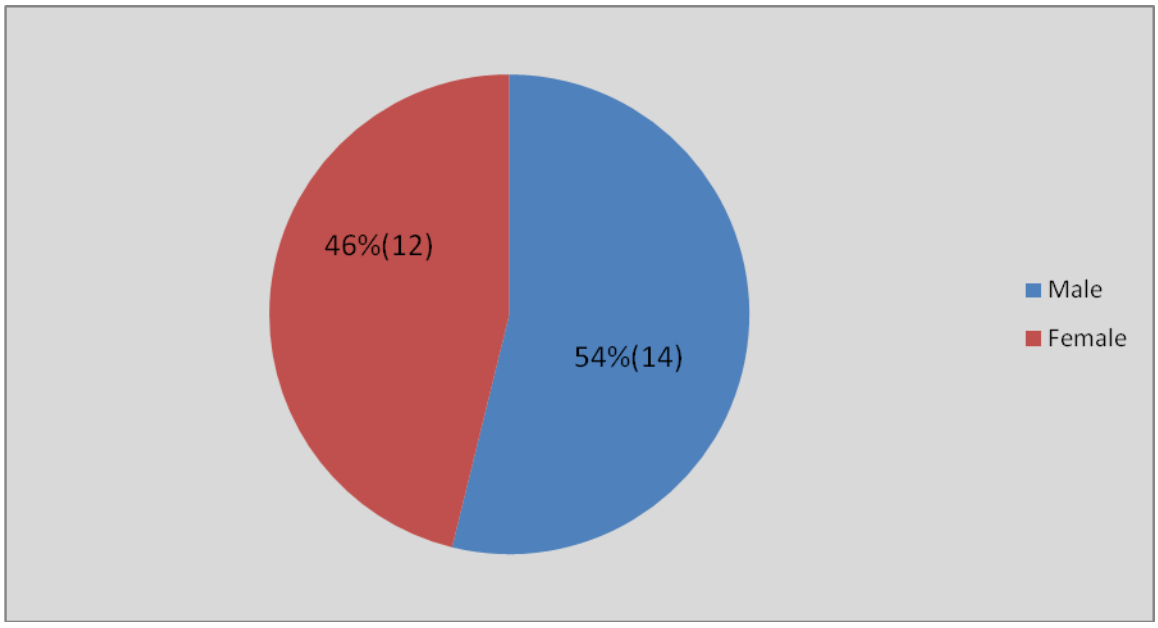


Figure 9:Gender distribution among adults.

Site:

It was found that 27(23.9%) cases of medulloblastoma arose in the cerebellar hemisphere and 86(76.1%) in the midline (Figure 10) Of those in the midline 21(24.4%) arose from the roof of the fourth ventricle, 27(31.4%) from the floor of the fourth ventricle, 22(25.6%) were seen to arise in the cerebellar vermis and in 16(18.6%) the exact site in the midline was not specified as shown in the (Figure 11)

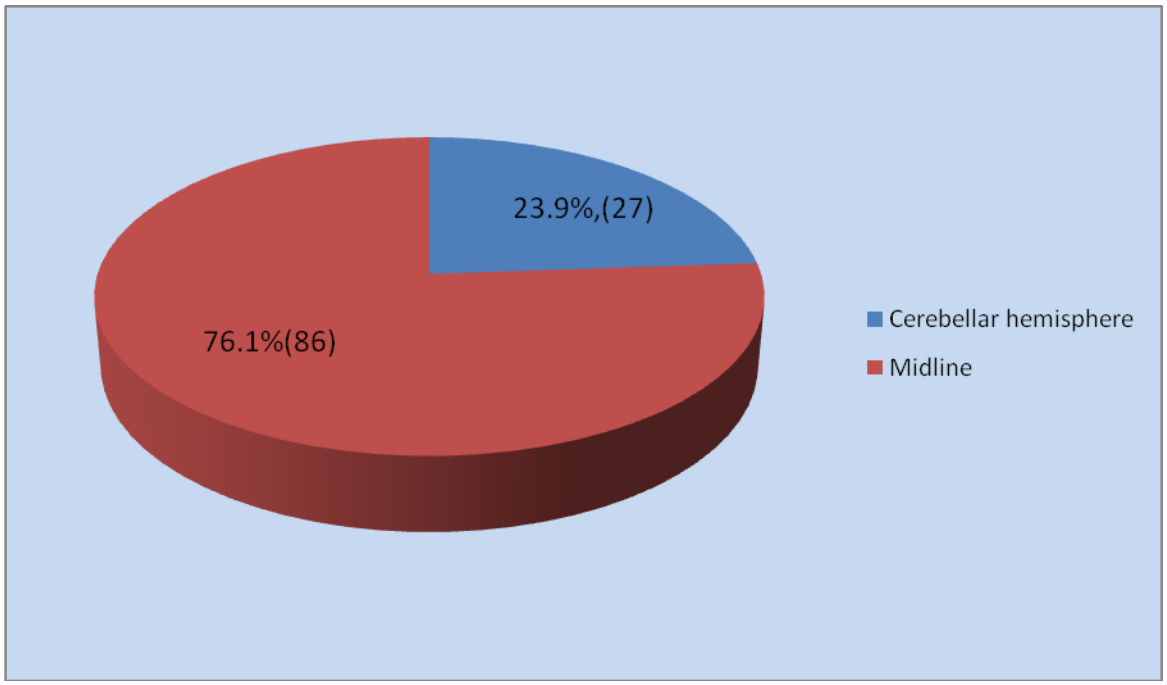


Figure 10:Distribution of medulloblastoma by site

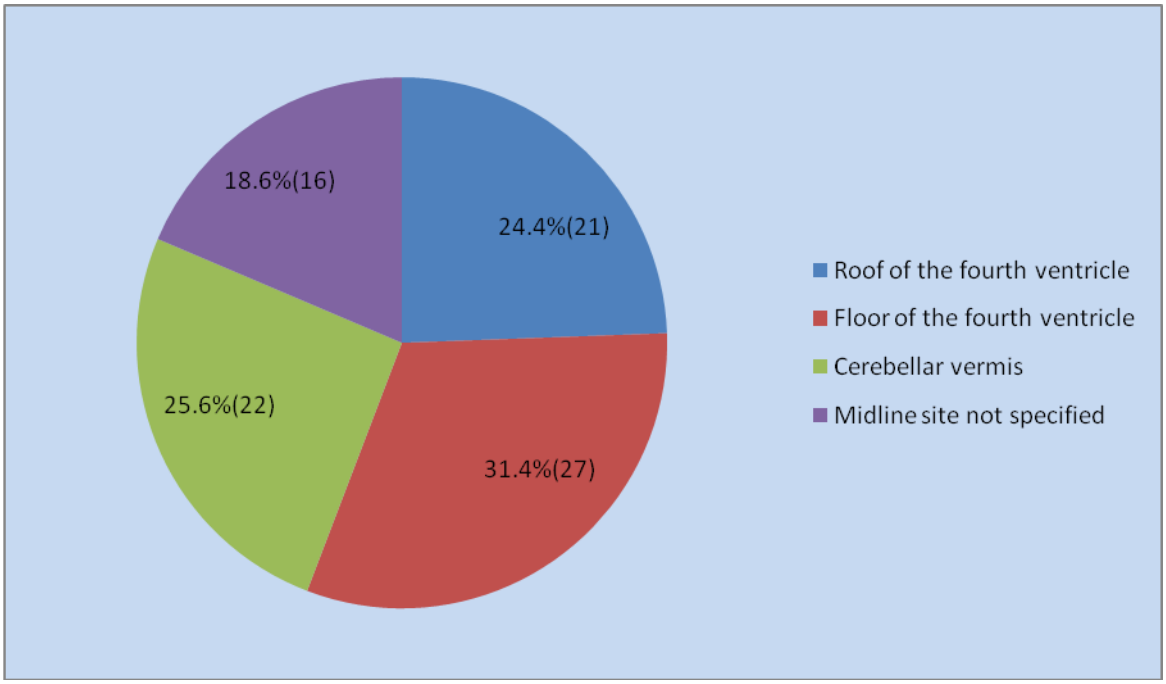


Figure 11: Distribution by subsite of midline medulloblastoma.

Histological subtypes:

We found all five histological subtypes of medulloblastoma in our study population. The predominant subtype corresponded to the Classic variant at 59.3% (67 cases). Desmoplastic medulloblastomas formed the next major group with 22.1 % (25 cases), followed by the Large Cell variant at 10.6% (12 cases). There were 6 (5%) and 3 (2.7%) of the Anaplastic and of Medulloblastoma with extensive nodularity (MBEN) subtypes respectively. (Figure 12)

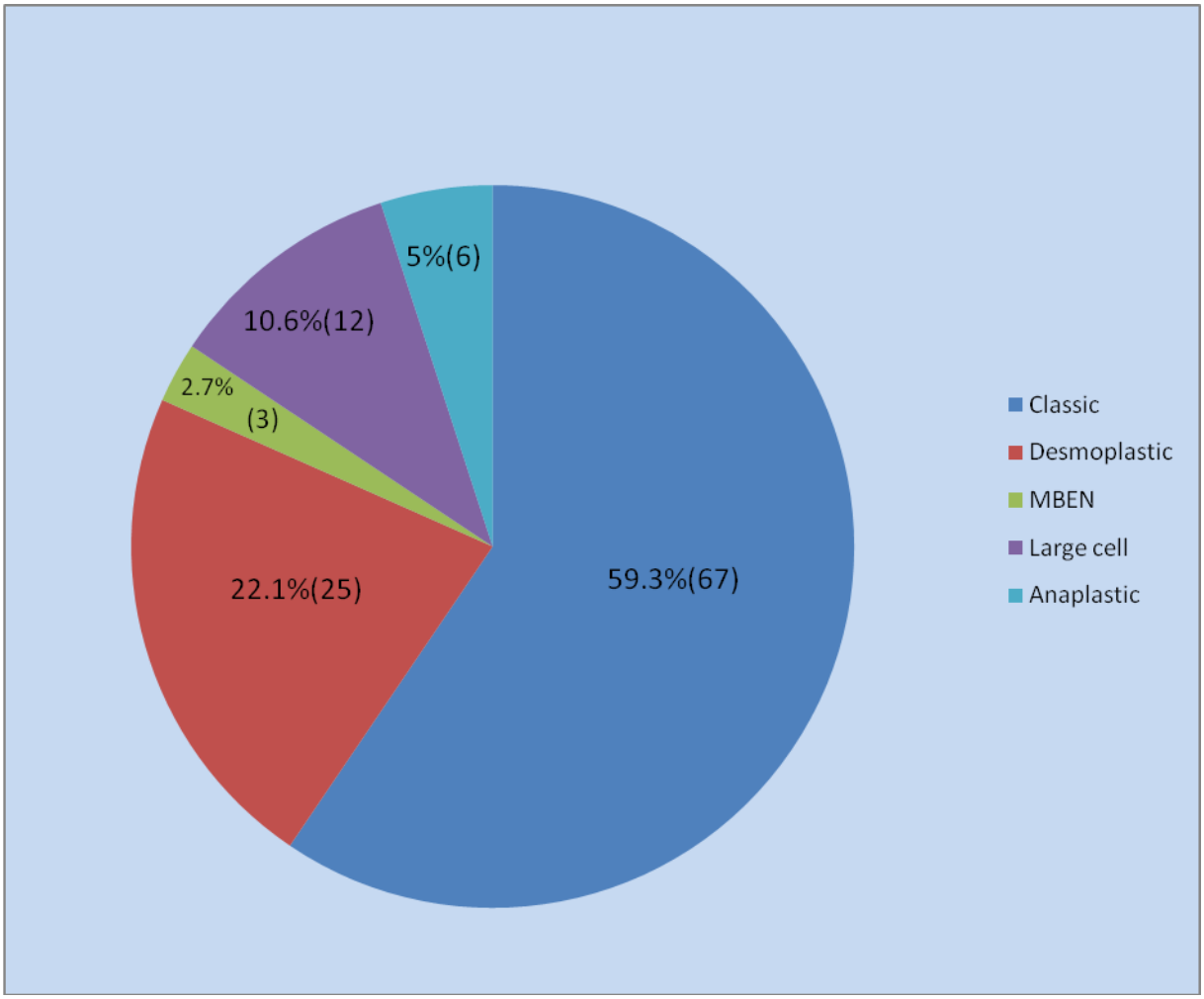


Figure 12: Distribution of cases by histological subtype in the study population.

In adults although Classic variant was the predominant subtype, the Desmoplastic variant came a close second constituting 46% and 35% of cases respectively (Figure 13). On the other hand in children, the Classic variant constituted nearly two thirds of the cases (66%) as opposed to only 19 % being Desmoplastic. (Figure 14). The age at presentation of the three cases of MBEN was 8 years, 10 years and 23 years. The proportion of adults and children in each subtype is as shown in Figure 15 .

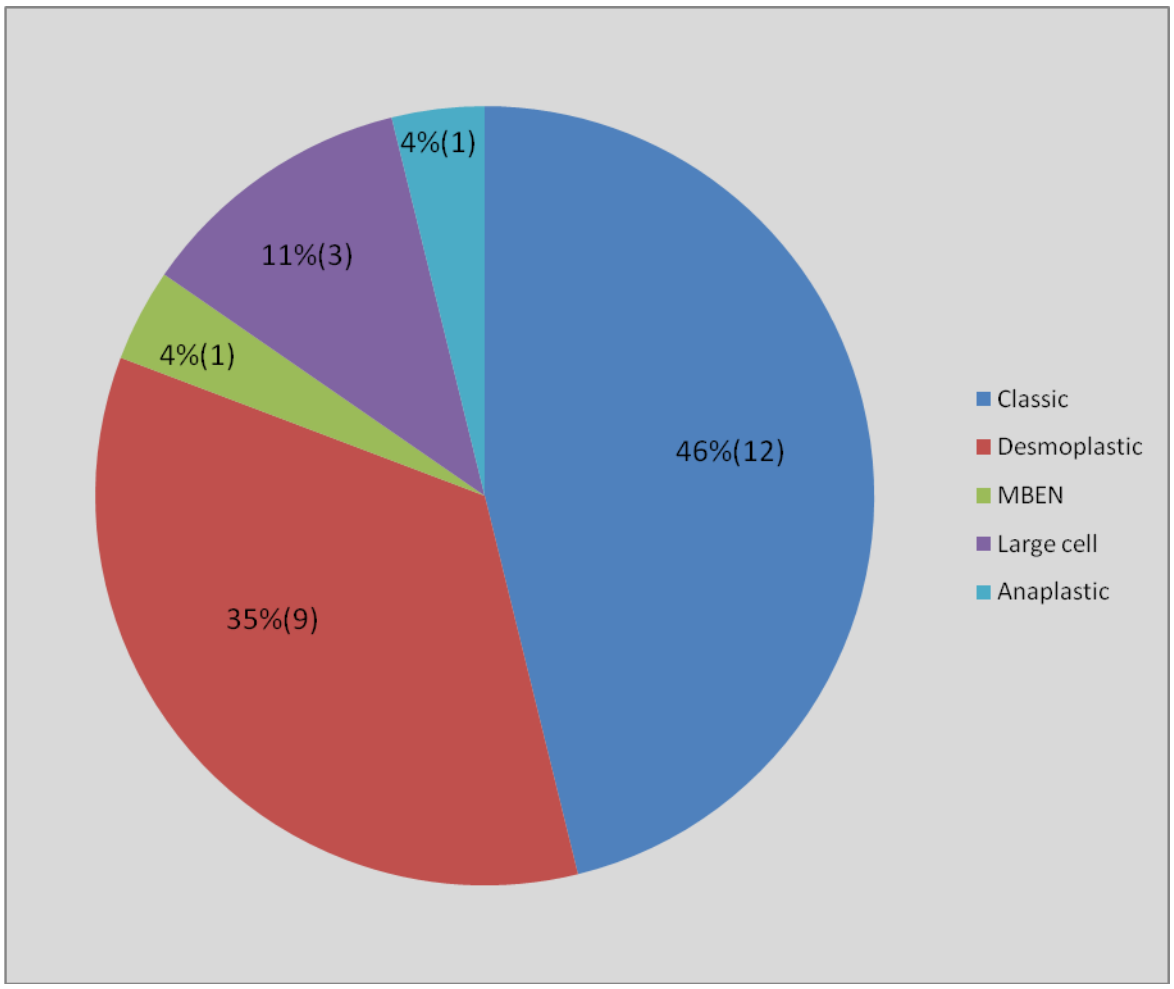


Figure 13: Distribution of cases by histological subtype in adults.

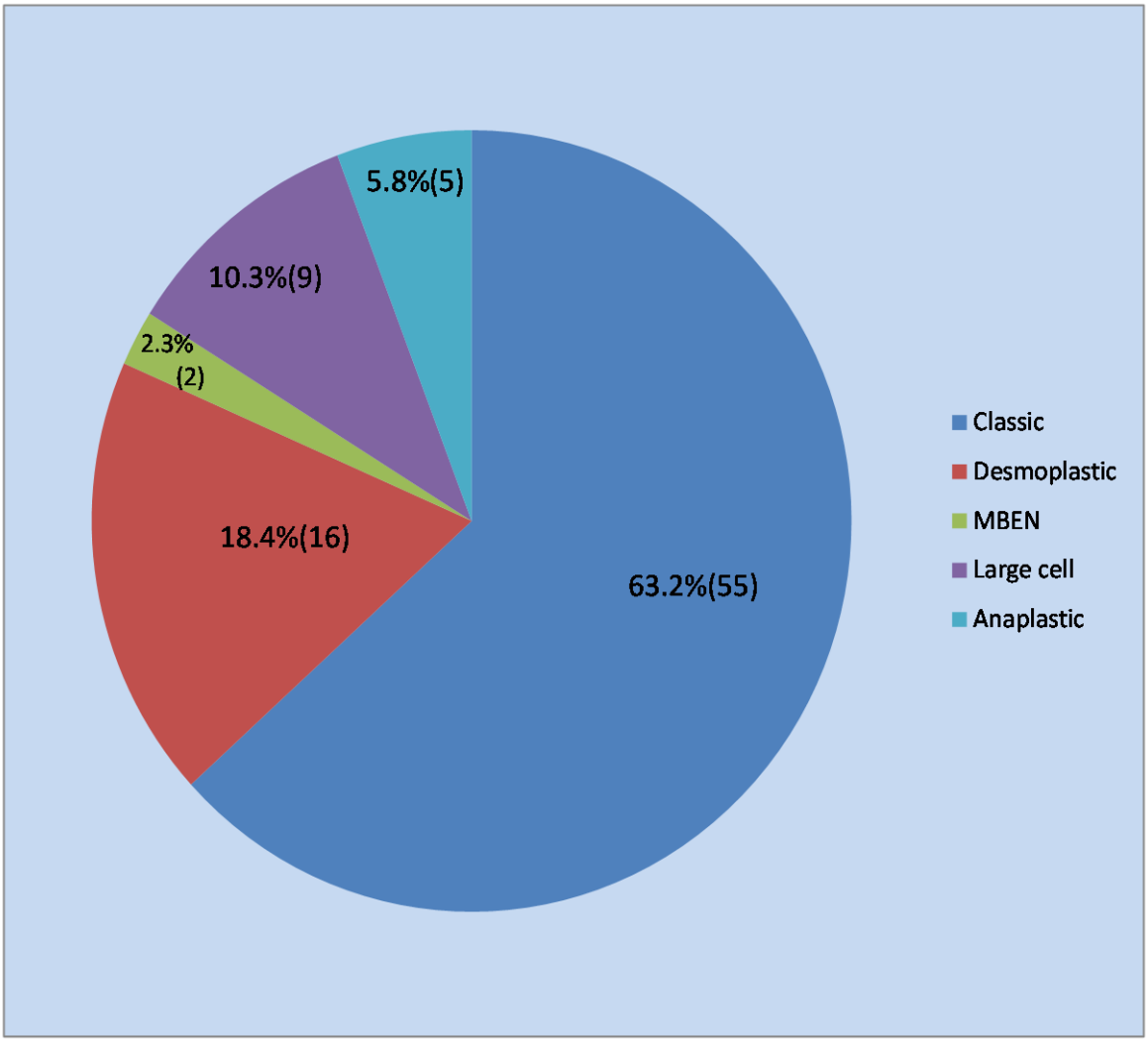


Figure 14: Distribution of cases by histological subtype in children.

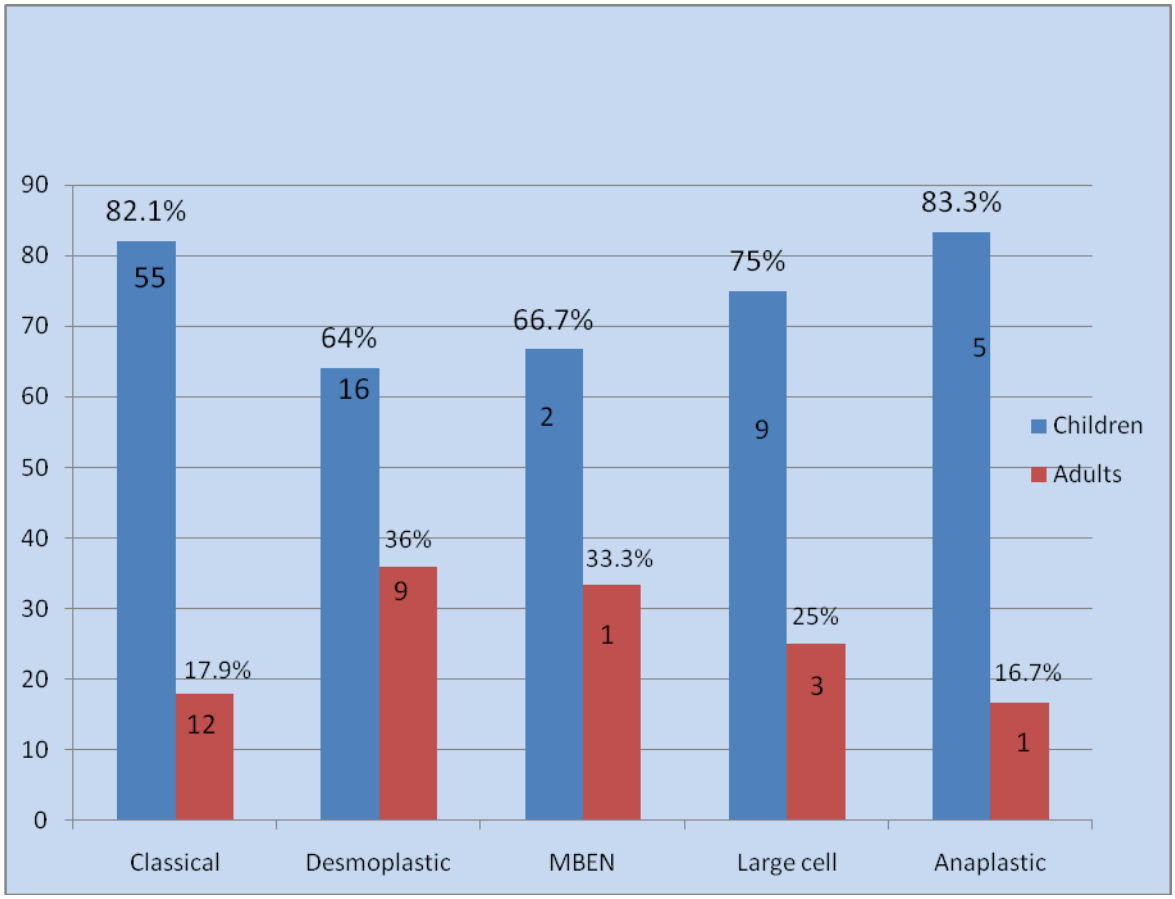


Figure 15: Distribution by age of the histological subtypes.

Of the 27 medulloblastomas seen in the cerebellar hemispheres, the majority, 59.3% were of the Desmoplastic variant (16 cases) and a quarter of the cases were of the Classic variant, 07/27 (25.9%) ($p<0.001$).The proportion of the other histological variants seen in the cerebellar hemispheres was as shown in Figure 16. Of 86 medulloblastoma in the midline 60 (69.8%) were of Classic histology ($p<0.001$) (Figure 17) .

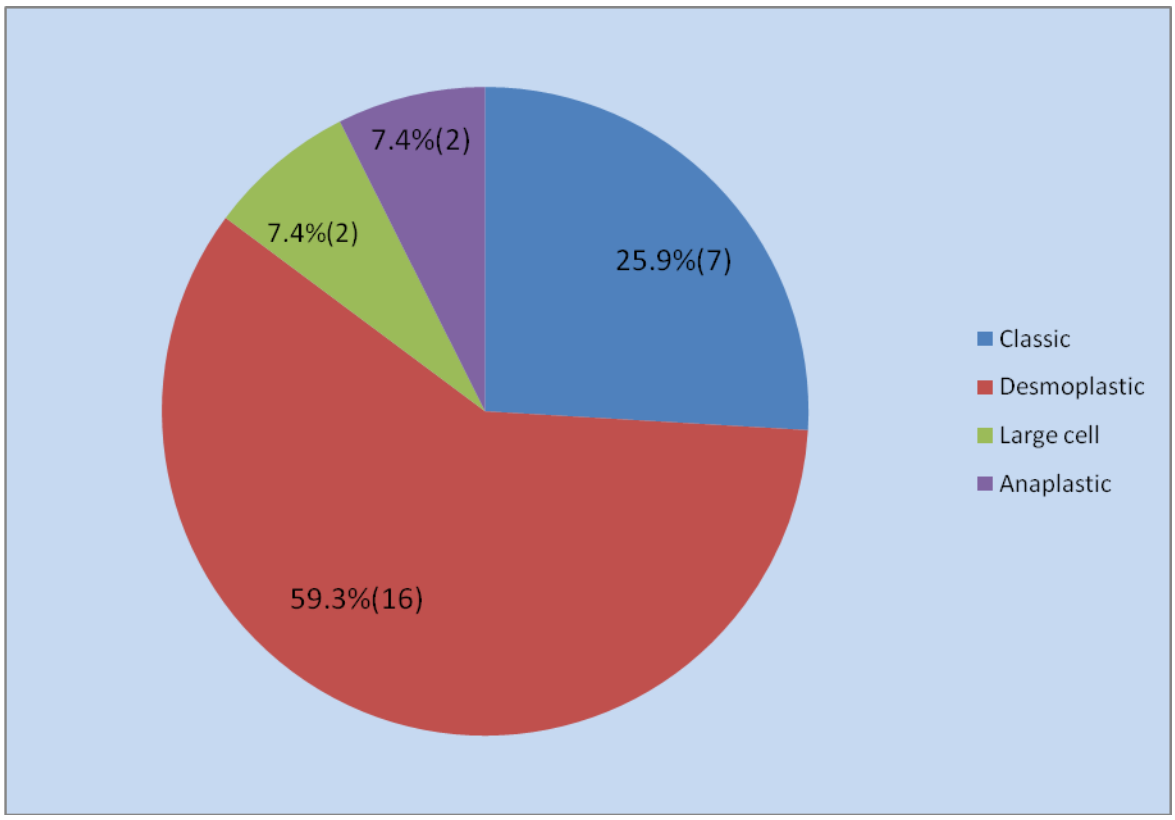


Figure 16:Distribution by histological subtype in the cerebellar hemisphere.

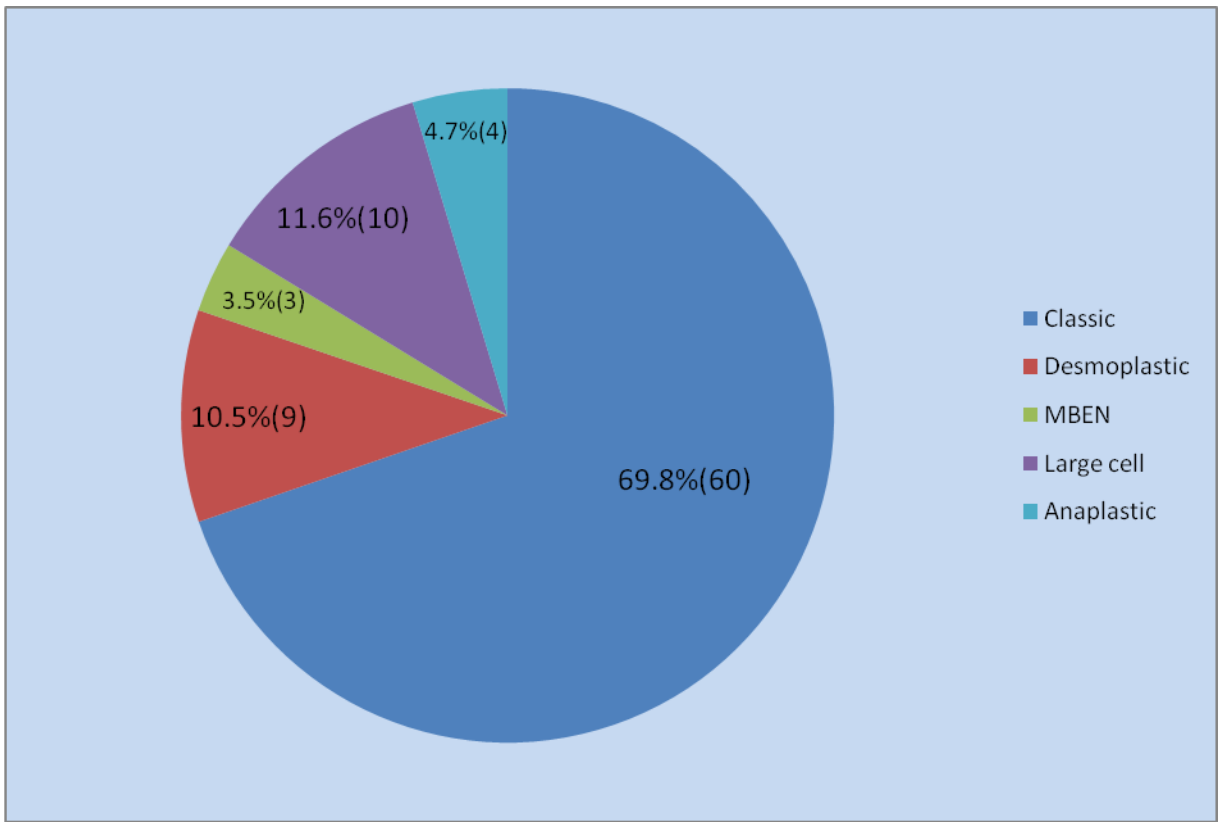


Figure 17: Distribution by histological subtype in the midline.

Amongst the Desmoplastic tumours, 57.1% occurred in the cerebellar hemisphere as shown in the Figure 18.

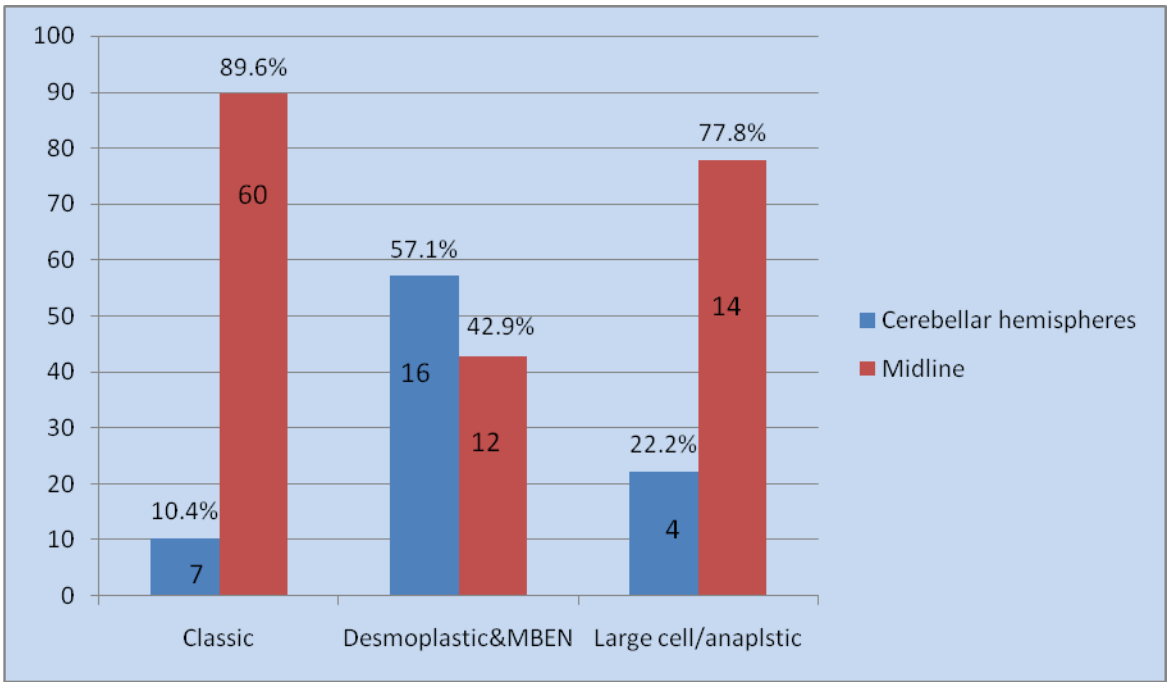


Figure 18: Distribution by histological subtype in the cerebellar hemisphere

Histological Features:

The Classic subtype was characterized by closely packed round to oval cells with hyper-chromatic (carrot shaped) nuclei surrounded by scant cytoplasm and high mitotic activity.(Figures 19(a)-19(i))

The Desmoplastic variant was characterized nodular pattern and reticulin free zones (pale islands) which were surrounded by densely packed cells with dense intercellular reticulin.(Figure 20a-20c.) Those cases with only dense collagenous and reticulin fibres without any nodular pattern were not classified as Desmoplastic or nodular variant.(Figure 20(d)&20(e))

The MBEN variant was characterized by expanded lobular architecture with enlarged reticulin free zones rich in neuropil like tissue.The tumour cells had uniform nuclei and clear cytoplasm resembling neurocytes.(Figure 21(a)-21(f)).

The Large Cell variant was characterized by monomorphous population of cells with large, round, vesicular nuclei, prominent nucleoli and variable amount of cytoplasm with abundant mitotic and apoptotic figures.(Figure 22(a)-22(c))

The Anaplastic variant was characterized by the presence of increased nuclear pleomorphism, nuclear moulding, cell-cell wrapping and high mitotic activity. Apoptosis was prominent in this variant. Presence of the above mentioned features in focal areas was not considered sufficient to diagnose an anaplastic variant.(Figure 23(a)&23(b))

There was one case with myogenic differentiation which was seen in a case of medulloblastoma, Classic variant. There were no cases with melanocytic differentiation. (Figure 24(a)-24(d))

Rosettes:

In the present study, rosettes were seen in 37 cases out of the total 113 cases (33.7%). (Figure 25(a&b)). Although rosettes were not restricted to any one subtype, nearly two thirds of the cases with rosettes were of Classic histology, and about 10% were of the Anaplastic variant.(Figure 26)

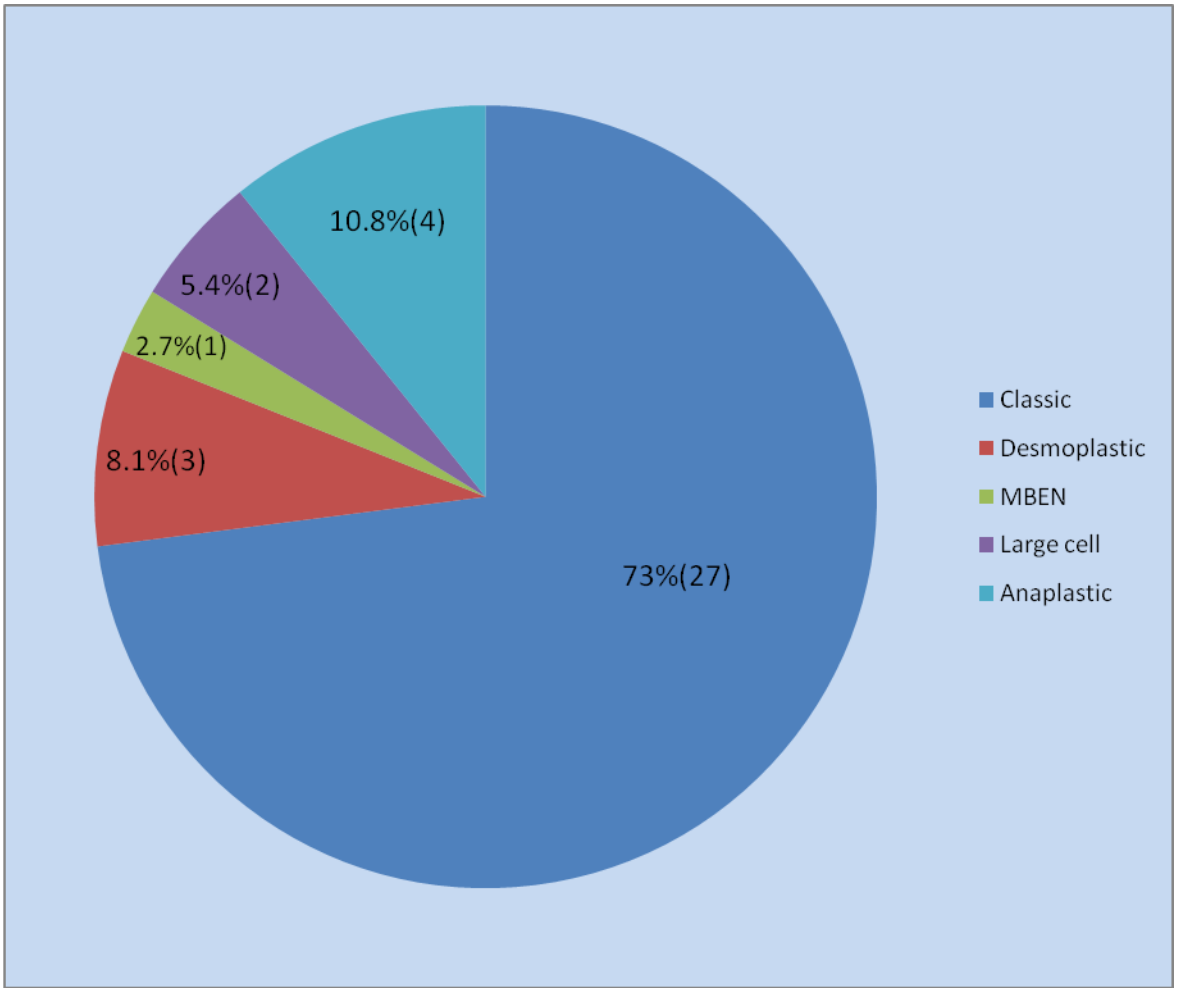


Figure 26: Prevalence of rosettes in the histological subtypes.

Nuclear moulding:

Nuclear moulding was present in 12 out of the 113 cases (10.6 %), with 05/12 cases (41.6%) being of the anaplastic variant.(Figure 27)

Cell to cell wrapping:

Of the total 113 cases of medulloblastoma, 10 cases (8.8 %) showed cell to cell wrapping with 05/10 cases(50%) being seen in the anaplastic variant.(Figure 28)

β-catenin immunoexpression:

β-catenin showed nuclear positivity in 23 cases(Figure 29(a)&29(b)) and both nuclear and cytoplasmic positivity in 27 cases.(Figure 29(c)) Cytoplasmic positivity in the absence of nuclear staining was seen in 7 cases and in 56 cases there was no immunostaining. (Figure 29(d)). Thus 44.2% (50) cases were immunopositive and 55.8% (63) cases were immunonegative for β-catenin.

Demographic profile and β-catenin immunoexpression:

β-catenin immunoexpression was seen in 78% children versus 22% of adults, this difference was statistically significant**.** ($p < 0.001$) (Figure 30)

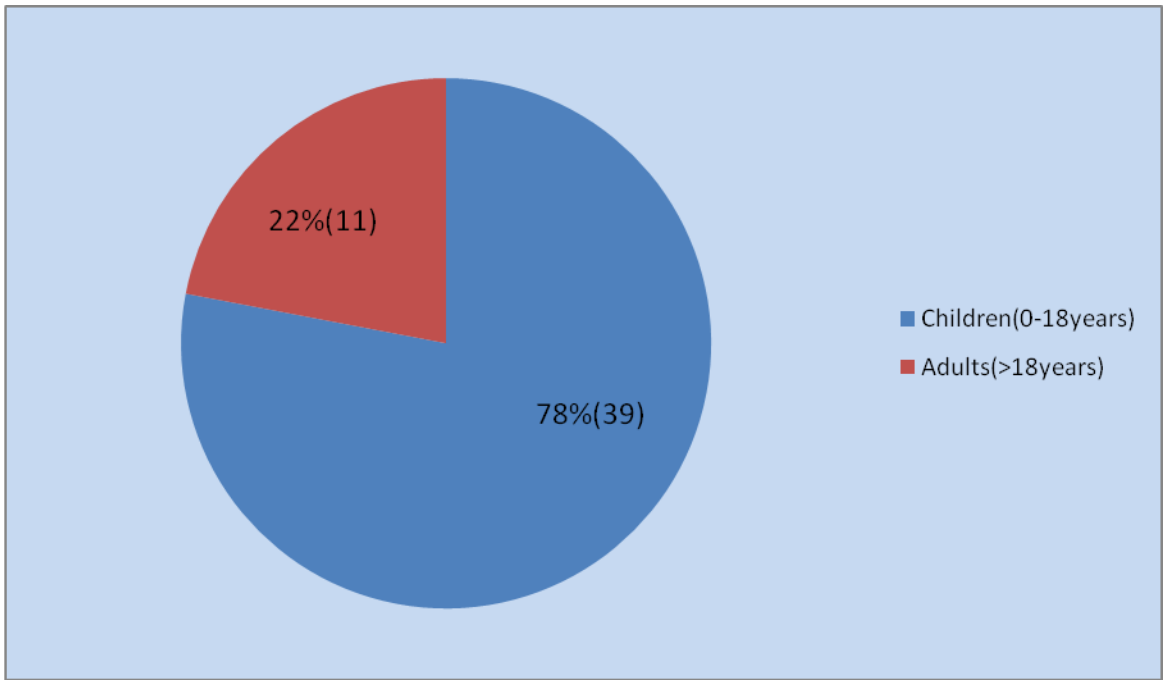


Figure 30(a): β-catenin immunoexpression among children and adults.

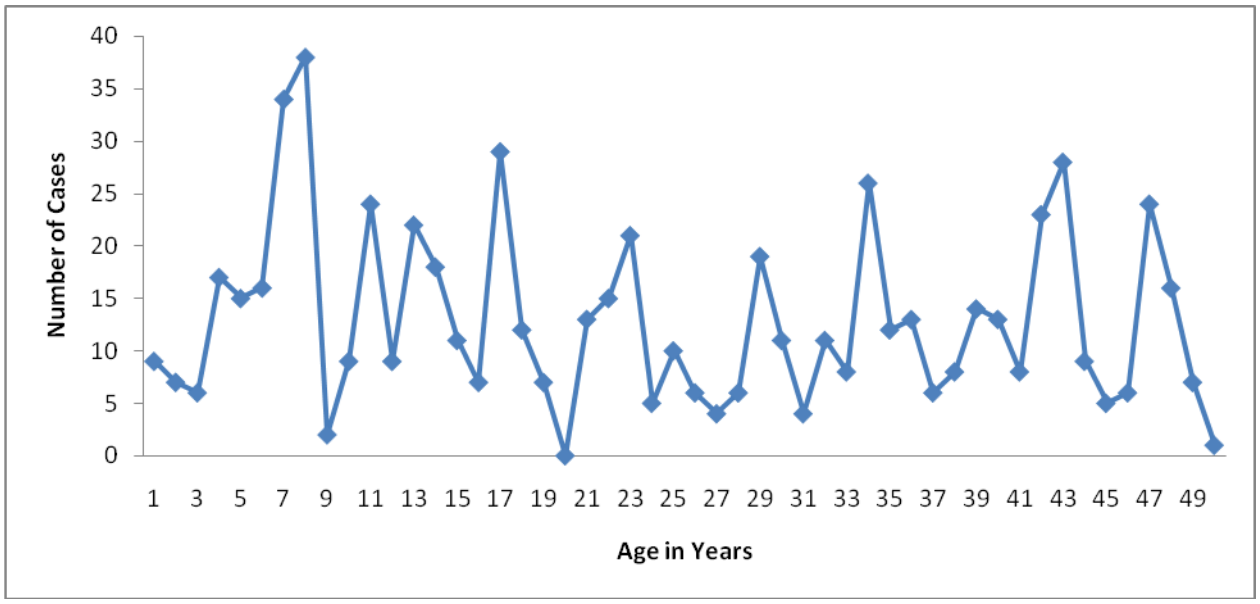


Figure 30(b) :Distribution by age in years of β-catenin immunoexpression

β-catenin immunoexpression was seen in 68% males versus 32% female, this difference was statistically significant. (p = 0.0007) (Figure 31)

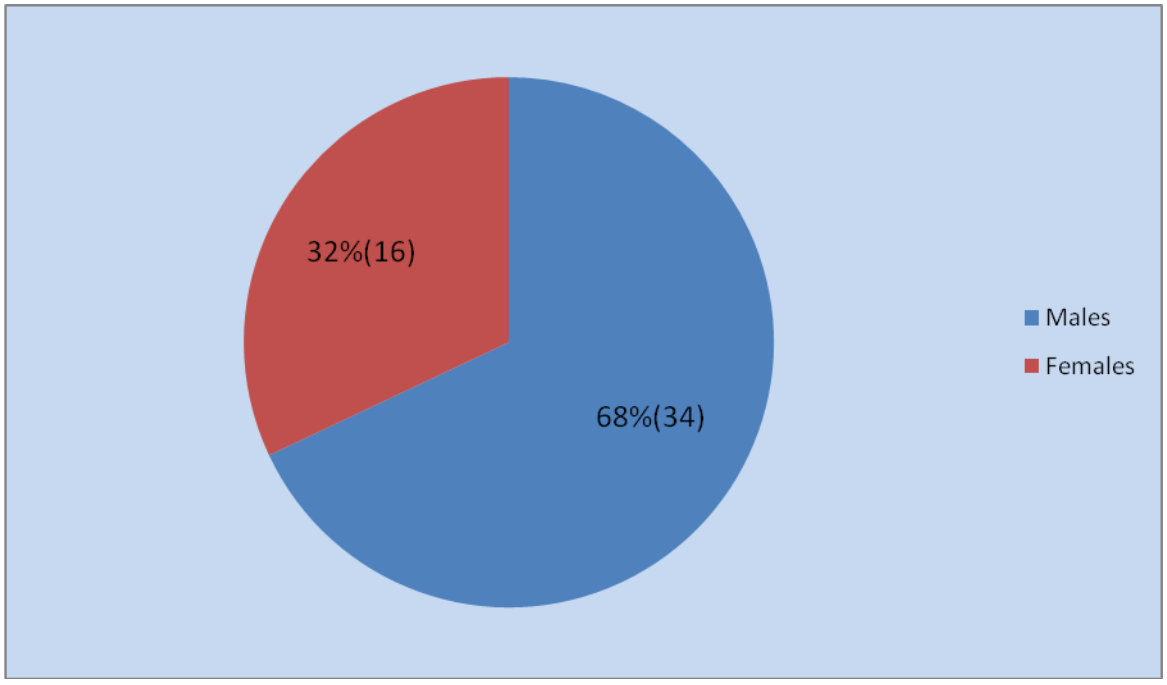


Figure 31.: β-catenin immunoexpression among males and females.

β-catenin immunoexpression and site :

Of the 27 tumours seen in the cerebellar hemispheres 22(81.5%) were negative for β-catenin. Of the tumours in the midline a little over 50% were positive for β-catenin.(Figure 32).

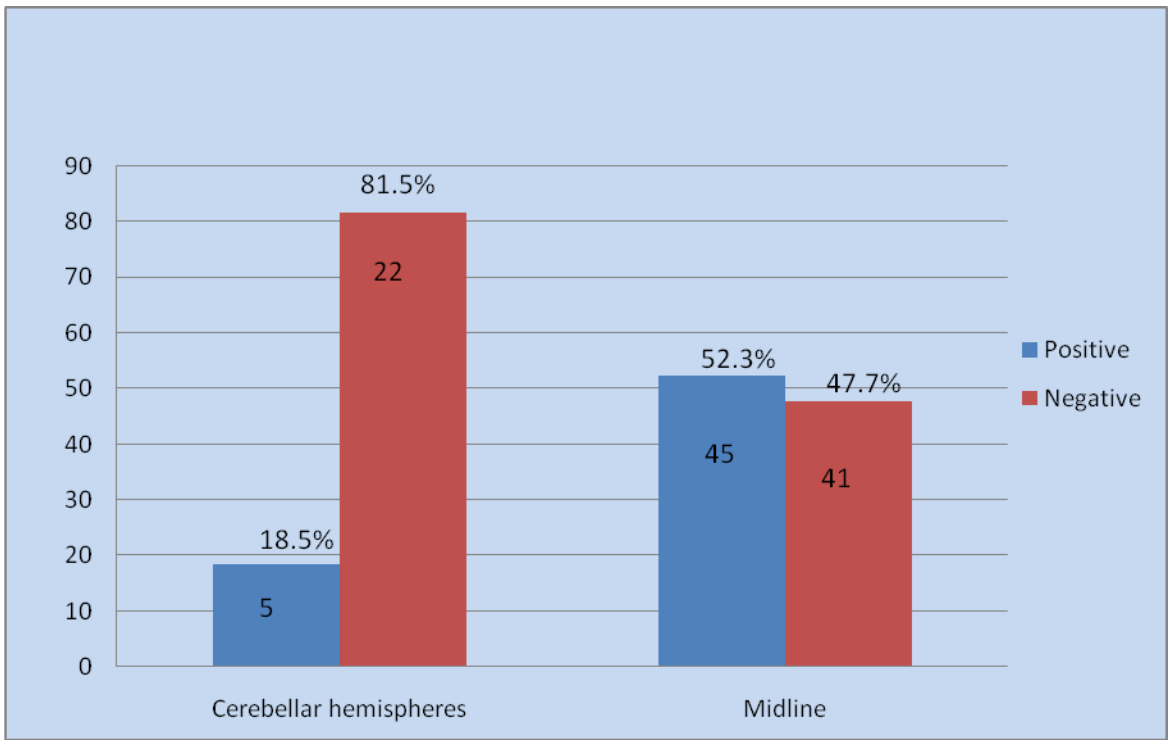


Figure 32:Distribution by β-catenin immunoexpression in the different sites.

On further analysis amongst the midline tumours, of those that arose from either the roof of the fourth ventricle or cerebellar vermis, 21 (48.8%) tumours were positive for β-catenin and 22 (51.2%) tumours were negative for β-catenin. Of those that arose in the floor of the fourth ventricle 18 (66.7%) were positive for β-catenin and 9 (33.3%) were negative for β-catenin (Figure 33). This difference was not found to be statistically significant (p value 0.22).

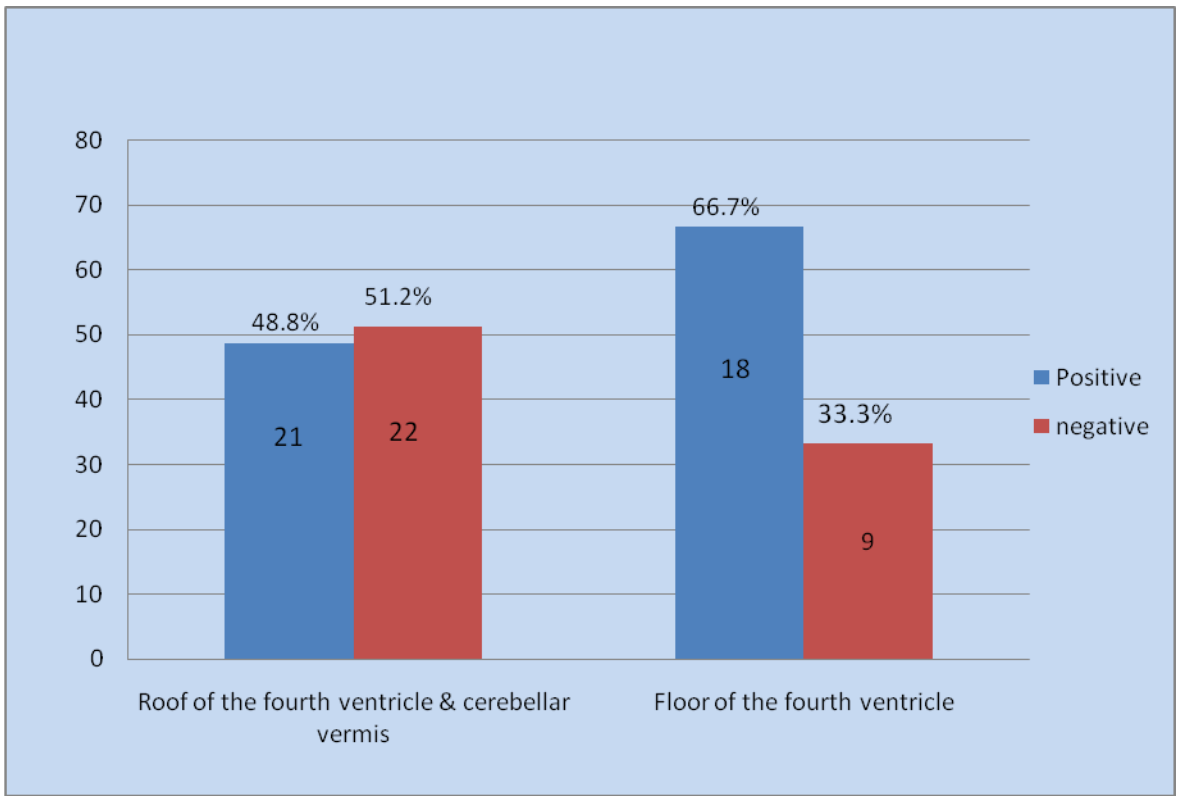


Figure 33: Distribution by β-catenin immunoexpression in the midline medulloblastomas.

β-catenin immunoexpression and histological subtypes:

The Classic variant variant constituted 88% of the β-catenin positive cases (Figure 34).

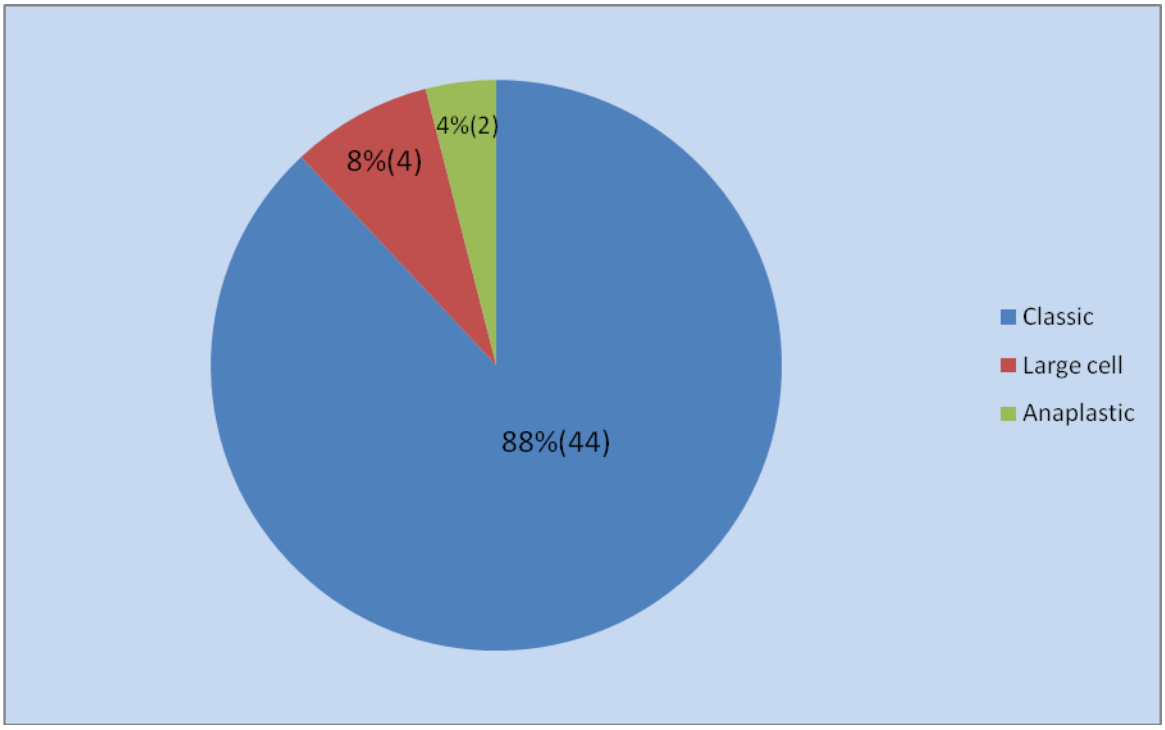


Figure 34: Prevalence of β-catenin positivity in the histological subtypes.

Among the five histological patterns β-catenin immunoexpression was seen in the majority of cases with Classic histology 65.7% (44 out of 67 cases) followed by Large Cell (4/12 cases) and Anaplastic variants (2/6 cases viz., 33.3% each). None of the Desmoplastic variant or Medulloblastoma with extensive nodularity were β-catenin positive. (Figure 35)

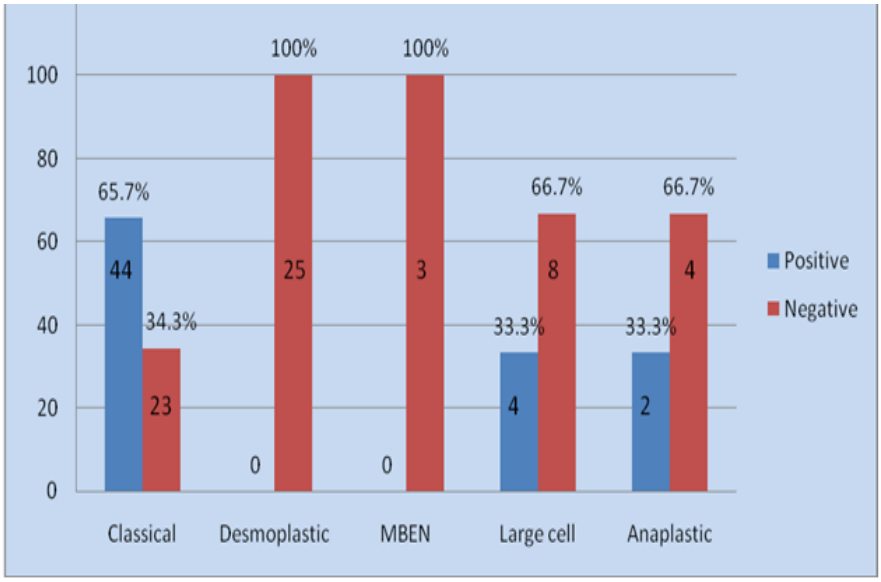


Figure 35: Distribution by β-catenin immunoexpression among the histological subtypes.

GAB-1 immunohistochemistry:

GAB-1 immunoexpression was seen in 23% (26) of cases(Figures 36(a)-36(c)).

Demographic profile and GAB-1 immunoexpression:

GAB-1 immunoexpression was seen in 61.5 % children versus 38.5% of adults, this difference was not statistically significant. (p = 0.16)(Figure 37(a&b)

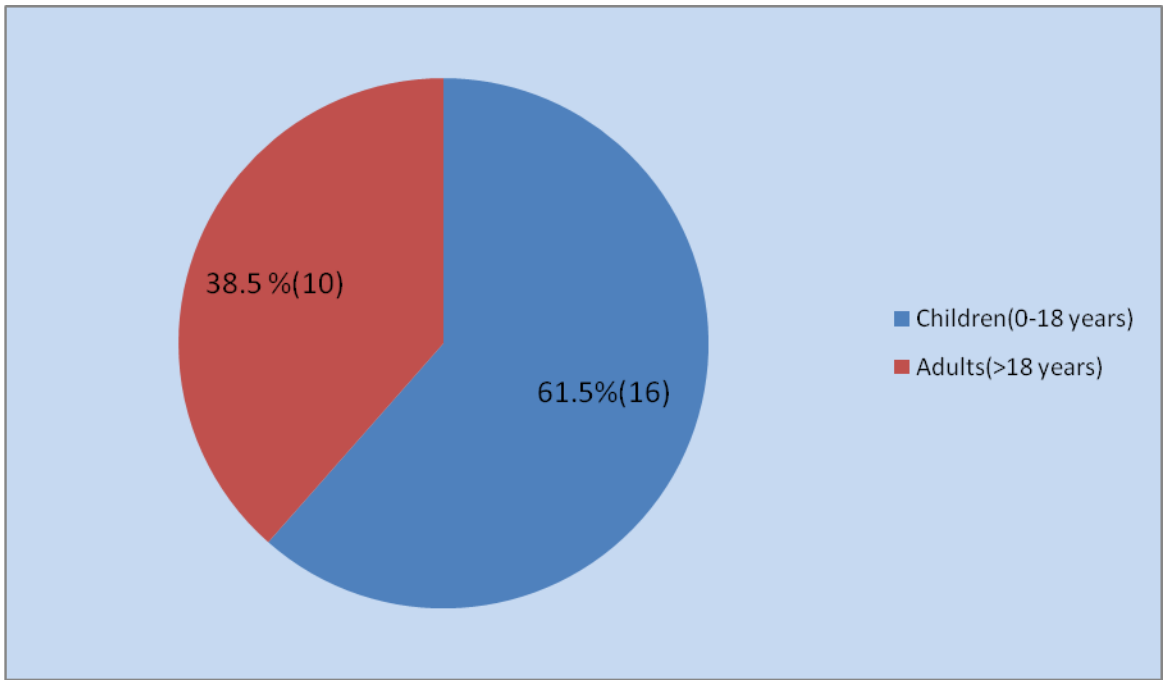


Figure 37(a): GAB-1 immunoexpression among children and adults.

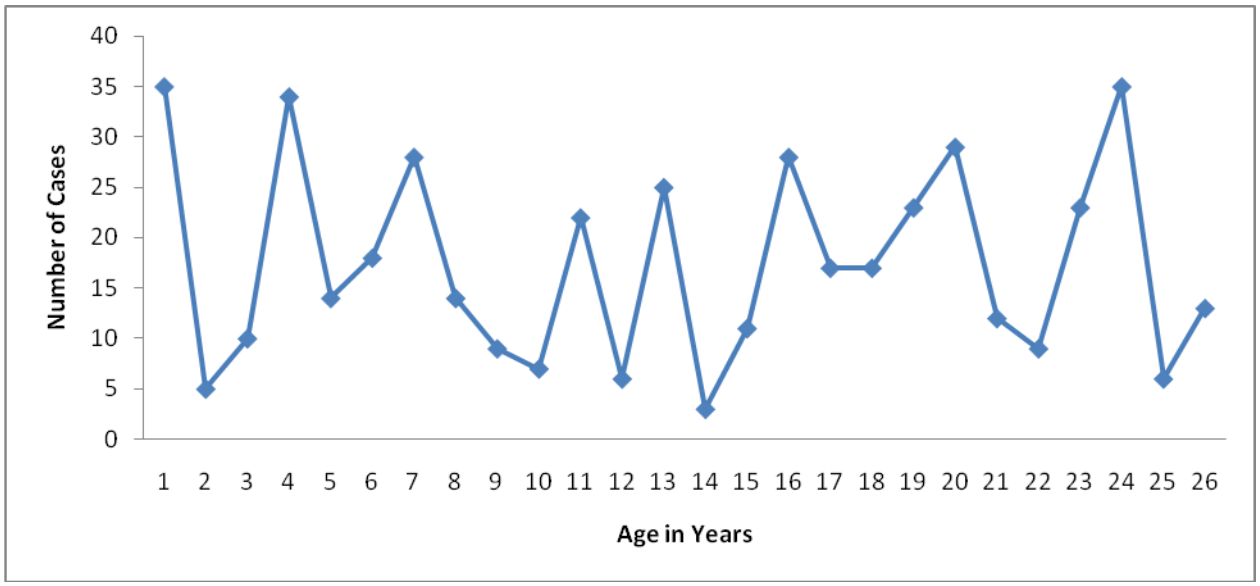


Figure 37(b): Distribution by age in years of GAB-1 immunoexpression

GAB1 immunoexpression was seen in 57.7% males versus 42.3% female, a difference that was not statistically significant. (p = 0.40)(Figure 38)

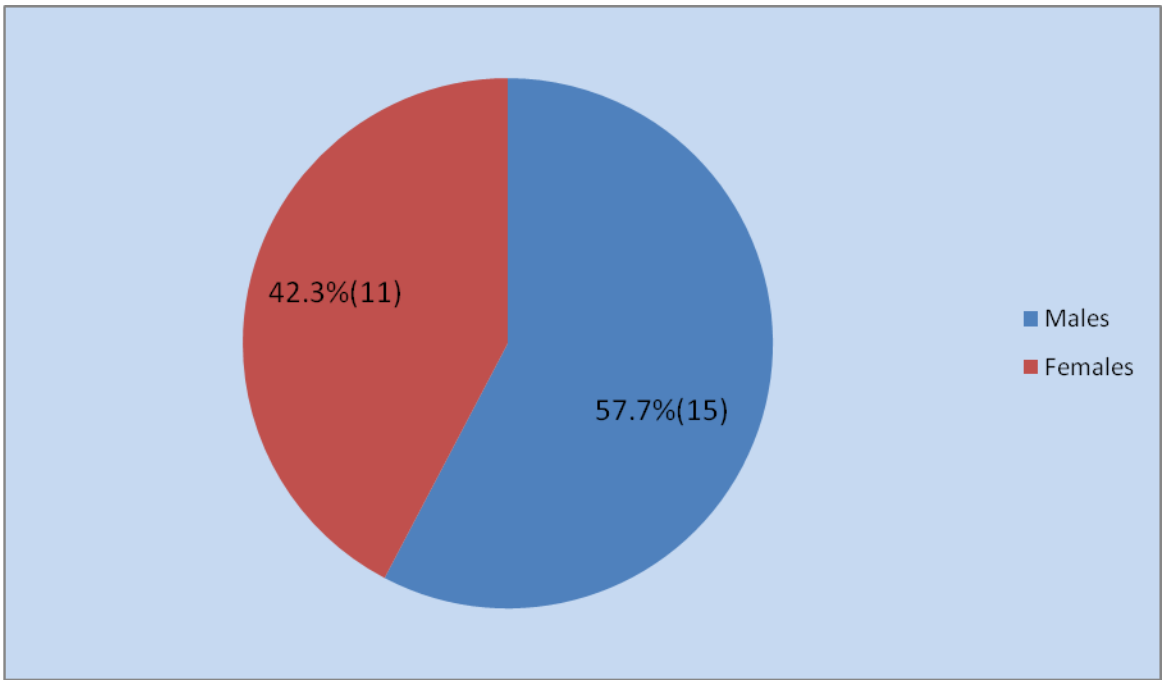


Figure 38:GAB-1 immunoexpression among males and females.

GAB-1 immunoexpression and site:

Of the 27 tumours that occurred in the cerebellar hemispheres 16 (59.1%) were immunopositive for GAB-1 (Figure 39). Of those tumours that occurred in the midline 76 88.4% (76 cases) were immunonegative for GAB-1. This difference was found to be statistically significant (p=0.002).

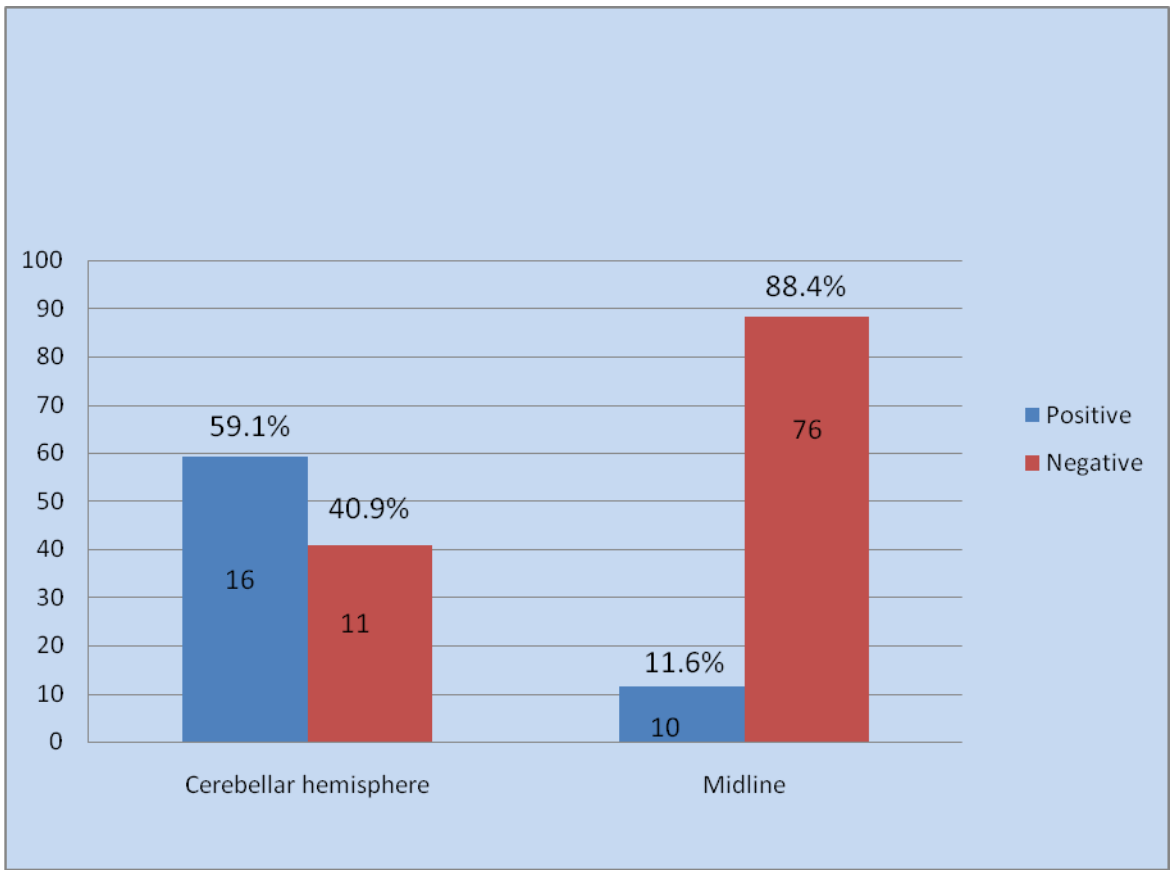


Figure 39:Distribution by GAB-1 immunoexpression in the different sites.

On further analysis amongst the midline tumours, of those that arose from either the roof of the fourth ventricle or cerebellar vermis only 04 (9.3%) were GAB-1 positive and amongst those that are from the floor of the fourth ventricle only 2(7.4%) were GAB-1 positive(Figure 40) sites.

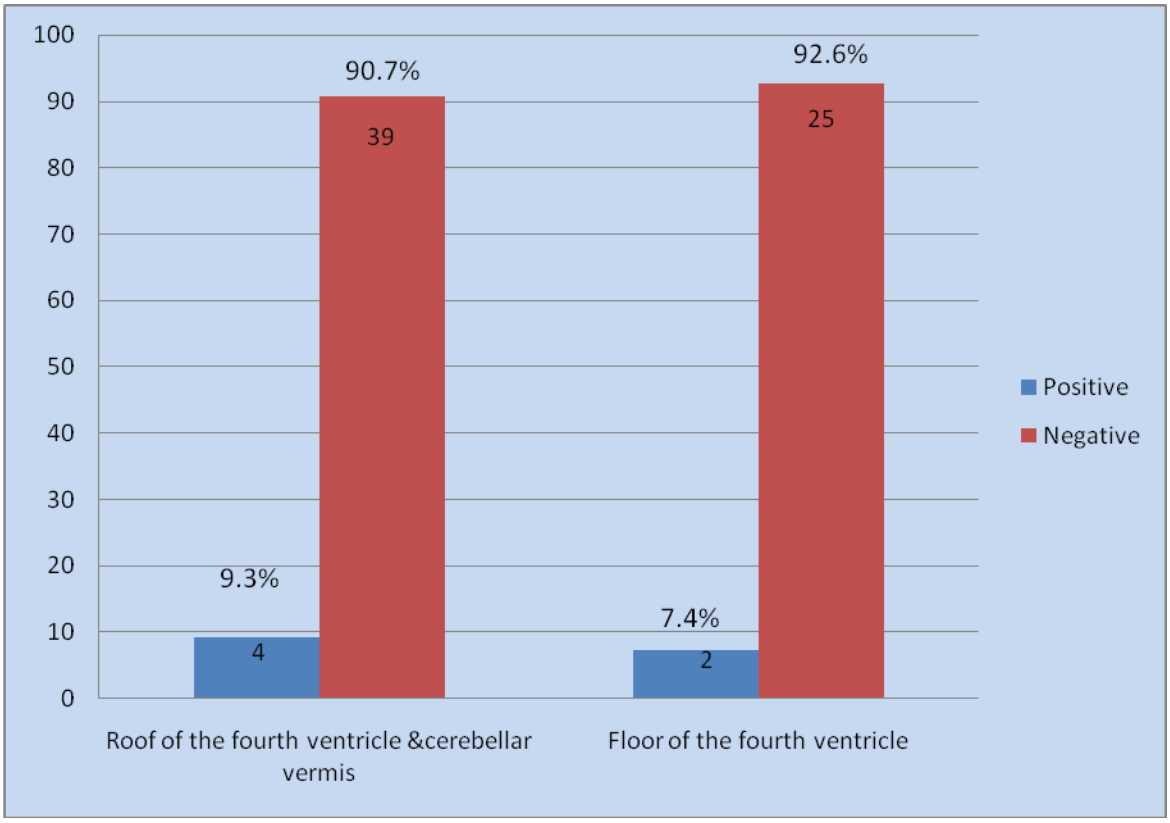


Figure 40: Distribution by GAB-1 immunoexpression in midline medulloblastoma.

GAB-1 immunoexpression and histological subtype

Nearly three fourth of the cases were of the Desmoplastic variant (19cases). (Figure 41) Amongst the Desmoplastic medulloblastomas, 76% (19/25) cases showed GAB-1 immunopositivity. (Figure 42)

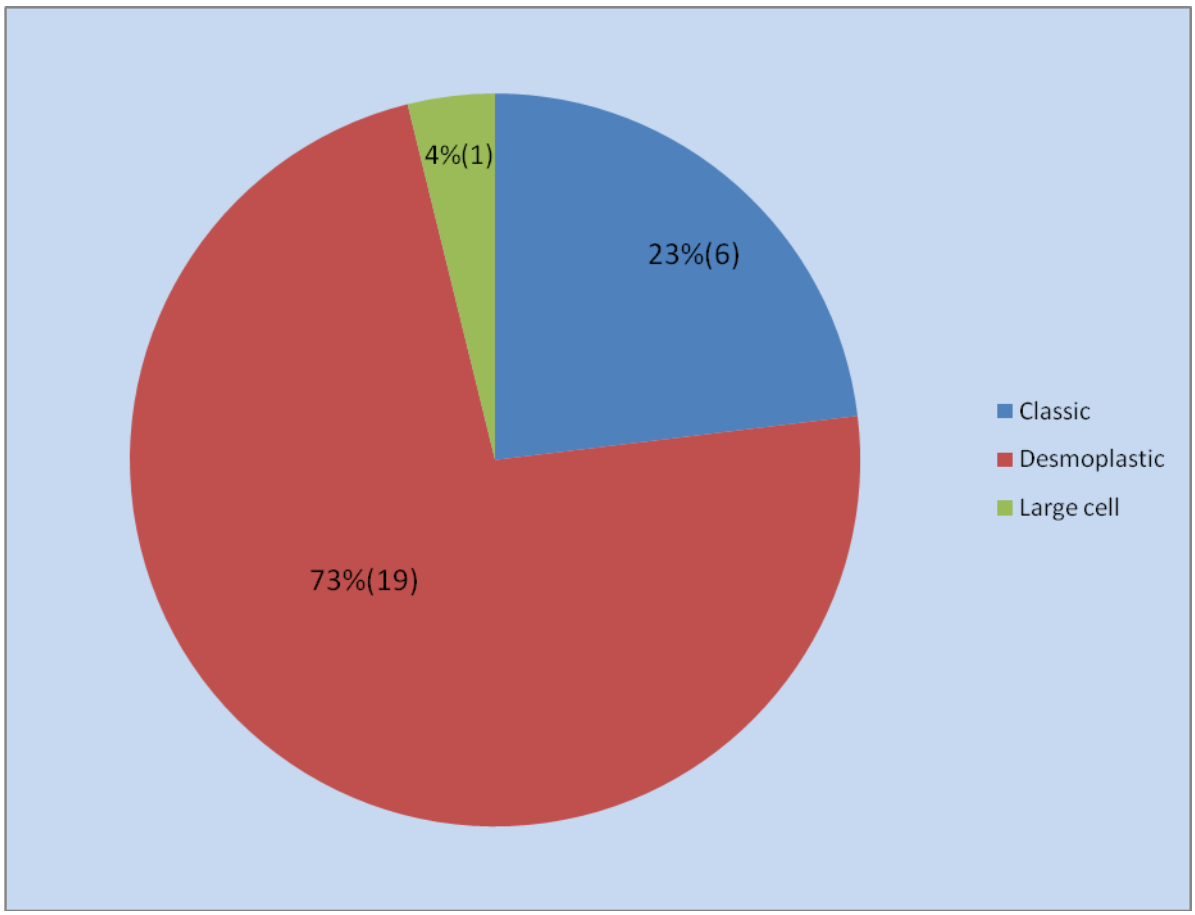


Figure 41: Prevalence of GAB-1 immunoexpression in the histological subtypes.

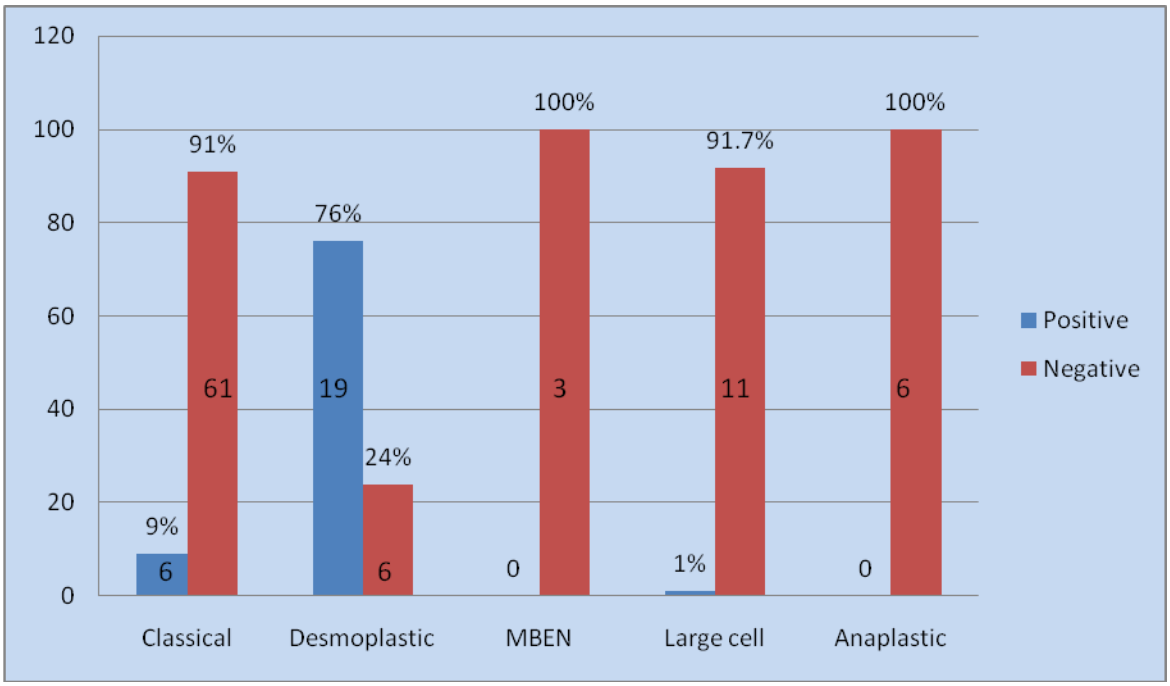


Figure 42 : Distribution by GAB-1 immunoexpression among the histological subtype.

Correlation between β-catenin and GAB-1:

The majority of GAB-1 positive cases were β-catenin negative (24/26). Of the two GAB-1 positive cases that were also β-catenin positive , 1 was of Desmoplastic histology and showed nuclear immunopositivity and the other was of Classic histology and showed both nuclear and cytoplasmic immunopositivity for β-catenin. There were 39 cases that were negative for both β-catenin and GAB-1.

Table 5: Comparison of β-catenin and GAB-1 immunoexpression

	β-catenin				Total
GAB-1	POSITIVE			NEGATIVE	
	CYTOPLASMIC	NUCLEAR	NUCLEAR + CYTOPLASMIC		
Positive	1	1	1	23	26
Negative	6	22	26	33	87
Total	7	23	27	56	113

NPR-3:

In the study population, only 2 cases (1.8%) showed NPR-3 positivity. Of the NPR-3 positive cases one was a Classic variant medulloblastoma and the other was a large cell variant of medulloblastoma.(Figure 43)

DISCUSSION

Medulloblastomas are high grade embryonal tumours of the central nervous system accounting for nearly 20% of childhood brain tumors and less than 1% of adult CNS tumors.(96) Although high grade, current management strategies have resulted in better long-term survival. (97) The 2007 WHO classification defines five histological subtypes of medulloblastoma, namely the Classic, Desmoplastic/nodular, Medulloblastoma with extensive nodularity, Anaplastic and Large Cell variants. (3) The developing cerebellum undergoes differentiation aided by signaling pathways such as Shh , Notch and WNT pathways .(63) A key mechanism involved, in tumorigenesis is believed to be dysregulation of some of these signaling pathways.(27) The wingless (WNT) and sonic hedgehog (Shh) signaling pathways are prime amongst those incriminated and several studies have identified the involvement of these two pathways in different subsets of medulloblastomas reliably and consistently. (98)

The present study was carried out with the aim of classifying adult and paediatric medulloblastomas, seen in this institution over a ten year period, into the histological subtypes as defined by WHO and to correlate the expression of markers of the WNT and SHH signaling pathways namely, β-catenin, GAB-1 and NPR-3, with these histological subtypes.

Medulloblastomas are 10 times more common in children when compared to adults. (99) In the present study more than three fourths of the medulloblastomas were seen in children. The mean age at diagnosis of the overall cohort in the present study was 13 years. The mean age was however lower at 9 years when considering only the paediatric medulloblastomas. The majority of the paediatric medulloblastomas in this study were seen between the ages of 5 and 14 years with the peak incidence being in the 8-11 years age group . Whilst other studies have reported a bimodal age distribution with peaks between 3-4 years and 8-9 years, we found a gradual increase in incidence from 2 years of age, peaking at 9 years and then gradually declining in incidence by age 18 years. (100) In present study, the mean age at diagnosis in adults was 26 years. This is contrast to the study by Giordana et al which reported a peak incidence in the age group of 15-19 years.(101) It is pertinent to note that the cut –off for pediatric age in the current study was 18 years and this might explain the difference in peak age at diagnosis in adults between our study and that of Giordana et al.(101) The reported incidence of adult medulloblastoma is upto 30%. In our study 23% of medulloblastomas were seen in adults.(100)

In the present study, a male preponderance was noted with twice as many males as females in the overall cohort. Amongst children, nearly two third of medulloblastomas were seen in males which is in keeping with the published literature (8). In contrast there was an almost equal gender distribution in adults. Giordana et al reported a male preponderance in adults.(101)

Medulloblastomas occur at two sites, the cerebellar hemispheres and in the midline from the cerebellar vermis projecting into the fourth ventricle and impinging on brain stem. (3) Most medulloblastomas arise in the midline. The tumors that are laterally situated are seen in older children, adolescents and adults. (77) In the present study over three fourths of the cases were seen in the midline.

Histological subtypes:

All the five histological subtypes as defined by the WHO were noted in this study. Among the five histological subtypes the Classic variant was found to be the predominant subtype constituting about 59.3%(67 cases) followed by the Desmoplastic variant 22.1% (25 cases) and the Large Cell variant at 10.6% (12 cases) . The prevalence of the different subtypes is similar to that reported by others .(102)

In adults, nearly a third of the cases were of the Desmoplastic variant in contrast to children, where the Desmoplastic variant constituted only 1/5th of the cases. The Desmoplastic variant has a higher prevalence in infants and adults. (77) There were no infants with Desmoplastic medulloblastoma in our study. Moreover, amongst the 16 cases of Desmoplastic medulloblastoma seen in the paediatric age group, the range was from 3-17 years with the mean age at diagnosis being 9.9 years.

Medulloblastoma with extensive nodularity, usually presents at the age of 3years or younger . (103) However, in the present study, of the 3 cases of MBEN, 2 were seen in children and one in an adult. The two children were aged 8 and 10 years. In a study by

Garre et al(104) on age – dependant occurrence of medulloblastoma, 2 of the 12 cases were seen at 4 years and 6 years. However, the remaining 10 were less than 3 years of age. Our finding that the MBEN variant is not restricted to adults is unusual and larger cohorts from the Indian subcontinent should be studied to determine if this is due to genetic variations.

Rosettes:

Homer Wright rosettes are described as a feature of medulloblastoma. Although not universally seen, at least 40% of medulloblastomas are reported to contain Homer Wright rosettes, particularly in the Classic variant. (68) In the present study Homer Wright rosettes were seen in 33.7% (37 cases). About two thirds of these cases were of the Classic variant and about 10.8% of the cases were of Anaplastic histology.

Wingless Pathway Tumors

The WNT/ β-catenin signaling pathway plays a role in regulating embryogenesis of the brain. WNT/ β-catenin signaling pathway activation when unregulated leads to upregulation of transcription and imbalance in cell proliferation resulting in tumor formation . (27)

Nuclear expression of β-catenin with activation of WNT signaling pathways are seen in 10% of the sporadic medulloblastomas.(100) In present study, 44.2% of cases showed immunopositivity β-catenin . This is much higher than that reported by several (73) (100) who found positivity for β-catenin in 5-15% of medulloblastomas. In a study by Ellison

et al published in 2005 on 109 medulloblastomas, nuclear positivity for β-catenin was found in 25% of cases. (72) . A similar prevalence was reported by Clifford et al. However, a more recent study by the Ellison et al on a larger series, reported a more modest prevalence of WNT subgroup at 14%. . In studies from India by Kaur et al (105) , activation of the WNT signaling pathway was seen in 9.8% and 5.2% of cases respectively.

WNT pathway activated tumours are of classic histology, occurs in non infants and have dual peak of incidence at 10 and 20 years.(26) They are equally distributed between both genders. (100) In the present study a peak incidence was seen at 7 years of age and 68% of those with activation of WNT pathway were males.

In present study , among the five histological patterns, β-catenin immunoexpression was seen in a majority of the cases with Classic histology 65.7% (44 out of 67 cases). None of the Desmoplastic variant or medulloblastoma with extensive nodularity were β-catenin positive. Subsets of both Classic and Large Cell variants have been found to have activation of the WNT signaling pathway.(100) In the current study 4 of 12 Large Cell variants and 2 of 6 and Anaplastic variants showed nuclear positivity for β-catenin.

Sonic Hedgehog Pathway Tumors:

Sonic hedgehog (Shh) is a ligand that is secreted by the Purkinje cells of the developing cerebellum and promotes the replication of cells in the external granular layer. Shh binds to PTCH receptors which normally function as inhibitors of the transmembrane protein

Smoothened (SMO). This results in derepression of SMO, which in turn results in activation of Gli transcription factors. Suppressor of Fused (SUFU) also inhibits Shh signaling. Mutational inactivation of PTCH, mutation of SUFU, activating mutations of SMO all lead to activation of the Shh/PTCH pathway. (106) Shh pathway activation has been reported in over 25% of medulloblastomas. (107) The Desmoplastic/nodular variant has a close association with PTCH mutations (108) and mutations of SMO and SUFU.

In the present study, GAB-1 immunoexpression was seen in 23% (26) of cases. The Shh sub group of tumors are seen in about 30% of medulloblastomas overall. (100) The Shh subgroup has a bimodal distribution, seen more commonly in children younger than 3 years and in patients older than 16 years. They have an equal gender distribution(100). In our study, 61% of GAB-1 positive cases were seen in children, however there was no increase in any particular age group of children. There was a slight male preponderance.

Nearly three fourths of the cases with GAB-1 expression were of the desmoplastic variant. This data is keeping with previous studies.(72) Amongst the desmoplastic medulloblastomas, 76% (19/25) cases showed GAB-1 immunopositivity compared to the study by Ellison et al ((72)) in which 54% of desmoplastic tumours were of the Shh subgroup .

In present study, most of the GAB-1 cases were β-catenin negative (24/26). A study by Min et al(7) showed 3 cases with both nuclear β-catenin and GAB-1 expression, but one these cases showed monosomy 6 which was helpful in including the case under WNT

subgroup. This overlap of expression of β-catenin and GAB-1 may represent the concurrent activation of both Shh and WNT pathways in the development of medulloblastomas.

A study by GIBSON et al (27) found that WNT subgroup and Shh subgroup are anatomically distinct. Medulloblastoma with WNT signaling pathway activation are found to be infiltrate the dorsal brainstem and those with Shh pathway activation are located in the cerebellar hemispheres. Recent work in mouse models has also reported the expression of WNT pathway target genes in the lower rhombic lip of the cerebellum at day 11.5 of embryonic development, and the dorsal brainstem at day 15.5. In the present study 59% of tumors that were localized to the cerebellar hemispheres were found to be immunopositive for GAB-1, indicative of Shh pathway activation.

We also found that of the 27 cases seen in relation to the floor of the fourth ventricle, 18 (66.6%) were positive for β-catenin , which is in keeping with the finding that developmentally, the lower rhombic lip of cerebellum and dorsal brain stem express WNT pathway genes.

Non-WNT/Shh Tumors

Group 3 and Group 4 tumors are considered in the non-WNT/SHh subgroup. (7) Genetic driver mutations in these are yet to be established. There were 39 cases belonging to the nonWNT/Shh subgroup in our study, comprising 34.5% of cases. Kaur et al(105) reported a prevalence of 44.6% of this sub group.

Group 3 medulloblastomas are common in children, have the least favorable outcomes and have an increased incidence of leptomeningeal dissemination. They account for 25% of all medulloblastomas(107) . This group is characterized by large cell/anaplastic histology, chromosome 7 gain, chromosome 8 loss and NPR-3 expression. Min et al found that NPR-3 lacked sensitivity in the diagnosis of Group 3 tumors as it showed positivity in only 2 cases and one of these cases belonged to the WNT subgroup with CTNNB1 mutation.(7)

In present study, only 2 cases(1.8%) showed NPR-3 positivity. One was a Classic variant and the other a Large Cell variant of medulloblastoma.

Molecular sub grouping has allowed for categorization of medulloblastomas in ways that have prognostic and therapeutic significance. The present study represents the first step in characterization of the cohort of cases seen in our institution. Apart from expanding the panel of markers to completely characterize this cohort, future studies will require incorporation of more detailed genomic platforms to segregate these tumors in appropriate subtypes. Studies aimed at correlating treatment protocols and outcome with these histological and molecular subtypes are warranted to optimize treatment protocols and for prognostication. While we utilized antibodies to GAB-1 and β-catenin to identify the two major subtypes of medulloblastomas, namely Shh and WNT, a larger panel of antibodies is required to better characterize this heterogeneous group of tumors.

Although histological subtyping of medulloblastomas has not been entirely supplanted, it is quite clear that histology alone is irrelevant in isolation and that identification of molecular sub groups is of paramount importance clinically. The challenge we face in the Indian setting, is to develop simple inexpensive techniques for sub- typing that can be adopted universally. Inter-center cooperation and setting up of core facilities for testing may be the way forward.

CONCLUSIONS

- Medulloblastomas are primarily tumors of childhood and 77% of the study population were children.
- The mean age at diagnosis of medulloblastomas was 13 years.
- The mean age amongst children was 9 years ,while in adults it was 26 years
- The M:F ratio of medulloblastomas was 2:1. In children there was clear male preponderance, whilst in adults there was a near equal gender distribution
- Medulloblastomas occurred predominantly in the midline with only a quarter arising laterally in the cerebellar hemisphere.
- The predominant histological subtype corresponded to the Classic variant.
- Desmoplastic medulloblastomas formed the next major histological variant, followed by the Large Cell variant
- Anaplastic and of Medulloblastoma with extensive nodularity (MBEN) subtypes formed $\leq$5% of medulloblastomas
- In adults, the Classic variant and the Desmoplastic variant had a nearly equal prevalence.
- In children, the Classic variant constituted nearly two thirds of the cases
- Medulloblastomas in the cerbellar hemispheres were predominantly of the Desmoplastic variant.
- Midline medulloblastomas were predominantly of the Classic variant.

- WNT signaling activation as evidenced by β-catenin immunoexpression was seen in 44.2% of medulloblastomas.
- WNT signaling activation was seen predominantly in males and children.
- Over 50% of midline medulloblastomas showed WNT signaling activation
- WNT signaling activation was seen in the majority of cases with Classic histology 65.7%, followed by Large Cell and Anaplastic variants
- None of the Desmoplastic variant or Medulloblastoma with extensive nodularity showed activation of the WNT signaling pathway.
- Shh signaling activation as evidenced by GAB-1 immunoexpression was seen in 23% of medulloblastomas.
- Shh signaling activation was seen predominantly in children, with a near equal gender distribution.
- Nearly 60% of medulloblastomas arising laterally in the cerebellar hemisphere showed Shh signaling activation
- Nearly three fourth of the cases with Shh signaling activation were of the Desmoplastic variant
- The non WNT/Shh sub-group constituted 34.5% of medulloblastomas.

LIMITATIONS

- Complete molecular sub-typing was not feasible as the battery of immunohistochemical markers was limited to three, owing to financial constraints.
- Treatment and Follow-up data was not obtained in the present study. This is required to ascertain true prognostic relevance of sub-typing.

BIBLIOGRAPHY

1. Wortham M, Jin G, Sun JL, Bigner DD, He Y, Yan H. Aberrant Otx2 Expression Enhances Migration and Induces Ectopic Proliferation of Hindbrain Neuronal Progenitor Cells. PLoS ONE. 2012 Apr 27;7(4):e36211.

2. Bien-Willner GA, López-Terrada D, Bhattacharjee MB, Patel KU, Stankiewicz P, Lupski JR, et al. Early recurrence in standard-risk medulloblastoma patients with the common idic(17)(p11.2) rearrangement. Neuro-Oncol. 2012 Jul;14(7):831–40.

3. Louis DN, Ohgaki H, Wiestler OD, Cavenee WK, Burger PC, Jouvet A, et al. The 2007 WHO classification of tumours of the central nervous system. Acta Neuropathol (Berl). 2007 Aug;114(2):97–109.

4. Pfister S, Remke M, Benner A, Mendrzyk F, Toedt G, Felsberg J, et al. Outcome prediction in pediatric medulloblastoma based on DNA copy-number aberrations of chromosomes 6q and 17q and the MYC and MYCN loci. J Clin Oncol Off J Am Soc Clin Oncol. 2009 Apr 1;27(10):1627–36.

5. Roussel MF, Hatten ME. Cerebellum development and medulloblastoma. Curr Top Dev Biol. 2011;94:235–82.

6. Leary SES, Olson JM. The molecular classification of medulloblastoma: driving the next generation clinical trials. Curr Opin Pediatr. 2012 Feb;24(1):33–9.

7. Min HS, Lee JY, Kim S-K, Park S-H. Genetic grouping of medulloblastomas by representative markers in pathologic diagnosis. Transl Oncol. 2013 Jun;6(3):265–72.

8. Robinson G, Parker M, Kranenburg TA, Lu C, Chen X, Ding L, et al. Novel mutations target distinct subgroups of medulloblastoma. Nature. 2012 Aug 2;488(7409):43–8.

9. Pećina-Šlaus N. Wnt signal transduction pathway and apoptosis: a review. Cancer Cell Int. 2010 Jun 30;10(1):22.

10. Ellison DW, Dalton J, Kocak M, Nicholson SL, Fraga C, Neale G, et al. Medulloblastoma: clinicopathological correlates of SHH, WNT, and non-SHH/WNT molecular subgroups. Acta Neuropathol (Berl). 2011 Mar;121(3):381–96.

11. Leary SES, Olson JM. The molecular classification of medulloblastoma: driving the next generation clinical trials. Curr Opin Pediatr. 2012 Feb;24(1):33–9.

12. Northcott PA, Korshunov A, Witt H, Hielscher T, Eberhart CG, Mack S, et al. Medulloblastoma comprises four distinct molecular variants. J Clin Oncol Off J Am Soc Clin Oncol. 2011 Apr 10;29(11):1408–14.

13. Kool M, Korshunov A, Remke M, Jones DTW, Schlanstein M, Northcott PA, et al. Molecular subgroups of medulloblastoma: an international meta-analysis of transcriptome, genetic aberrations, and clinical data of WNT, SHH, Group 3, and Group 4 medulloblastomas. Acta Neuropathol (Berl). 2012 Apr;123(4):473–84.

14. Northcott PA, Korshunov A, Pfister SM, Taylor MD. The clinical implications of medulloblastoma subgroups. Nat Rev Neurol. 2012 Jun;8(6):340–51.

15. Das P, Puri T, Suri V, Sharma MC, Sharma BS, Sarkar C. Medulloblastomas: a correlative study of MIB-1 proliferation index along with expression of c-Myc, ERBB2, and anti-apoptotic proteins along with histological typing and clinical outcome. Childs Nerv Syst ChNS Off J Int Soc Pediatr Neurosurg. 2009 Jul;25(7):825–35.

16. Kunder R, Jalali R, Sridhar E, Moiyadi A, Goel N, Goel A, et al. Real-time PCR assay based on the differential expression of microRNAs and protein-coding genes for molecular classification of formalin-fixed paraffin embedded medulloblastomas. Neuro-Oncol. 2013 Dec;15(12):1644–51.

17. Robles P de, Fiest KM, Frolkis AD, Pringsheim T, Atta C, Germaine-Smith CS, et al. The worldwide incidence and prevalence of primary brain tumors: a systematic review and meta-analysis. Neuro-Oncol. 2015 Jun 1;17(6):776–83.

18. Gupta T, Shirsat N, Jalali R. Molecular Subgrouping of Medulloblastoma: Impact Upon Research and Clinical Practice. Curr Pediatr Rev. 2015;11(2):106–19.

19. McKinney PA. Central nervous system tumours in children: epidemiology and risk factors. Bioelectromagnetics. 2005;Suppl 7:S60–8.

20. Kool M, Korshunov A, Pfister SM. Update on molecular and genetic alterations in adult medulloblastoma. Memo. 2012 Sep;5(3):228–32.

21. Yu J, Shi WE, Zhao R, Gao X, Li H. Epidemiology of brain tumors in children aged two and under: A 10-year single-institute study. Oncol Lett. 2015 Apr;9(4):1651–6.

22. Sarkar C, Deb P, Sharma MC. Recent advances in embryonal tumours of the central nervous system. Childs Nerv Syst ChNS Off J Int Soc Pediatr Neurosurg. 2005 Apr;21(4):272–93.

23. Smoll NR, Drummond KJ. The incidence of medulloblastomas and primitive neurectodermal tumours in adults and children. J Clin Neurosci Off J Neurosurg Soc Australas. 2012 Nov;19(11):1541–4.

24. Sarkar C, Jain A, Sharma M, Suri V, Kale S, Mahapatra A, et al. Spectrum of pediatric brain tumors in India: A multi-institutional study. Neurol India. 2011;59(2):208.

25. Asirvatham JR, Deepti AN, Chyne R, Prasad MSN, Chacko AG, Rajshekhar V, et al. Pediatric tumors of the central nervous system: a retrospective study of 1,043 cases from a tertiary care center in South India. Childs Nerv Syst ChNS Off J Int Soc Pediatr Neurosurg. 2011 Aug;27(8):1257–63.

26. Massimino M, Giangaspero F, Garrè ML, Gandola L, Poggi G, Biassoni V, et al. Childhood medulloblastoma. Crit Rev Oncol Hematol. 2011 Jul;79(1):65–83.

27. Gibson P, Tong Y, Robinson G, Thompson MC, Currle DS, Eden C, et al. Subtypes of medulloblastoma have distinct developmental origins. Nature. 2010 Dec 23;468(7327):1095–9.

28. Grammel D, Warmuth-Metz M, von Bueren AO, Kool M, Pietsch T, Kretzschmar HA, et al. Sonic hedgehog-associated medulloblastoma arising from the cochlear nuclei of the brainstem. Acta Neuropathol (Berl). 2012 Apr;123(4):601–14.

29. Pizer BL, Clifford SC. The potential impact of tumour biology on improved clinical practice for medulloblastoma: progress towards biologically driven clinical trials. Br J Neurosurg. 2009 Aug;23(4):364–75.

30. Halperin EC, Watson DM, George SL. Duration of symptoms prior to diagnosis is related inversely to presenting disease stage in children with medulloblastoma. Cancer. 2001 Apr 15;91(8):1444–50.

31. Turcot J, Despres JP, St Pierre F. Malignant tumors of the central nervous system associated with familial polyposis of the colon: report of two cases. Dis Colon Rectum. 1959 Oct;2:465–8.

32. Li FP, Fraumeni JF. Rhabdomyosarcoma in children: epidemiologic study and identification of a familial cancer syndrome. J Natl Cancer Inst. 1969 Dec;43(6):1365–73.

33. Raffel C, Jenkins RB, Frederick L, Hebrink D, Alderete B, Fults DW, et al. Sporadic medulloblastomas contain PTCH mutations. Cancer Res. 1997 Mar 1;57(5):842–5.

34. Paraf F, Jothy S, Van Meir EG. Brain tumor-polyposis syndrome: two genetic diseases? J Clin Oncol Off J Am Soc Clin Oncol. 1997 Jul;15(7):2744–58.

35. Taylor MD, Mainprize TG, Rutka JT, Becker L, Bayani J, Drake JM. Medulloblastoma in a child with Rubenstein-Taybi Syndrome: case report and review of the literature. Pediatr Neurosurg. 2001 Nov;35(5):235–8.

36. Fleck BJ, Pandya A, Vanner L, Kerkering K, Bodurtha J. Coffin-Siris syndrome: review and presentation of new cases from a questionnaire study. Am J Med Genet. 2001 Feb 15;99(1):1–7.

37. Rogers L, Pattisapu J, Smith RR, Parker P. Medulloblastoma in association with the Coffin-Siris syndrome. Childs Nerv Syst ChNS Off J Int Soc Pediatr Neurosurg. 1988 Feb;4(1):41–4.

38. Ryzhova MV, Zheludkova OG, Kumirova ÉV, Shishkina LV, Panina TN, Gorelyshev SK, et al. [Characteristics of medulloblastoma in children under age of three years]. Zhurnal Vopr Neĭrokhirurgii Im N N Burdenko. 2013;77(1):3–10; discussion 11.

39. Swartling FJ, Grimmer MR, Hackett CS, Northcott PA, Fan Q-W, Goldenberg DD, et al. Pleiotropic role for MYCN in medulloblastoma. Genes Dev. 2010 May 15;24(10):1059–72.

40. Northcott PA, Nakahara Y, Wu X, Feuk L, Ellison DW, Croul S, et al. Multiple recurrent genetic events converge on control of histone lysine methylation in medulloblastoma. Nat Genet. 2009 Apr;41(4):465–72.

41. Grandori C, Cowley SM, James LP, Eisenman RN. The Myc/Max/Mad network and the transcriptional control of cell behavior. Annu Rev Cell Dev Biol. 2000;16:653–99.

42. Rubinfeld B, Souza B, Albert I, Müller O, Chamberlain SH, Masiarz FR, et al. Association of the APC gene product with beta-catenin. Science. 1993 Dec 10;262(5140):1731–4.

43. Nicholson J, Wickramasinghe C, Ross F, Crolla J, Ellison D. Imbalances of chromosome 17 in medulloblastomas determined by comparative genomic hybridisation and fluorescence in situ hybridisation. Mol Pathol MP. 2000 Dec;53(6):313–9.

44. Gilbertson R, Wickramasinghe C, Hernan R, Balaji V, Hunt D, Jones-Wallace D, et al. Clinical and molecular stratification of disease risk in medulloblastoma. Br J Cancer. 2001 Sep 1;85(5):705–12.

45. Bunt J, Hasselt NE, Zwijnenburg DA, Koster J, Versteeg R, Kool M. Joint Binding of OTX2 and MYC in Promotor Regions Is Associated with High Gene Expression in Medulloblastoma. PLoS ONE [Internet]. 2011 Oct 10 [cited 2015 Jun 9];6(10). Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3189962/

46. Bai R-Y, Staedtke V, Lidov HG, Eberhart CG, Riggins GJ. OTX2 Represses Myogenic and Neuronal Differentiation in Medulloblastoma Cells. Cancer Res. 2012 Nov 15;72(22):5988–6001.

47. Gottardo NG, Hansford JR, McGlade JP, Alvaro F, Ashley DM, Bailey S, et al. Medulloblastoma Down Under 2013: a report from the third annual meeting of the International Medulloblastoma Working Group. Acta Neuropathol (Berl). 2014 Feb;127(2):189–201.

48. Nüsslein-Volhard C, Wieschaus E. Mutations affecting segment number and polarity in Drosophila. Nature. 1980 Oct 30;287(5785):795–801.

49. Mohn F, Weber M, Rebhan M, Roloff TC, Richter J, Stadler MB, et al. Lineage-specific polycomb targets and de novo DNA methylation define restriction and potential of neuronal progenitors. Mol Cell. 2008 Jun 20;30(6):755–66.

50. Kenney AM, Cole MD, Rowitch DH. Nmyc upregulation by sonic hedgehog signaling promotes proliferation in developing cerebellar granule neuron precursors. Dev Camb Engl. 2003 Jan;130(1):15–28.

51. Carlotti CG, Smith C, Rutka JT. The molecular genetics of medulloblastoma: an assessment of new therapeutic targets. Neurosurg Rev. 2008 Oct;31(4):359–68; discussion 368–9.

52. Ruiz i Altaba A. Catching a Gli-mpse of Hedgehog. Cell. 1997 Jul 25;90(2):193–6.

53. Ruiz i Altaba A, Palma V, Dahmane N. Hedgehog-Gli signalling and the growth of the brain. Nat Rev Neurosci. 2002 Jan;3(1):24–33.

54. Dahmane N, Ruiz i Altaba A. Sonic hedgehog regulates the growth and patterning of the cerebellum. Dev Camb Engl. 1999 Jun;126(14):3089–100.

55. Wechsler-Reya RJ, Scott MP. Control of neuronal precursor proliferation in the cerebellum by Sonic Hedgehog. Neuron. 1999 Jan;22(1):103–14.

56. Ellison DW, Clifford SC, Gajjar A, Gilbertson RJ. What's new in neuro-oncology? Recent advances in medulloblastoma. Eur J Paediatr Neurol EJPN Off J Eur Paediatr Neurol Soc. 2003;7(2):53–66.

57. Goodrich LV, Milenković L, Higgins KM, Scott MP. Altered neural cell fates and medulloblastoma in mouse patched mutants. Science. 1997 Aug 22;277(5329):1109–13.

58. Su LK, Vogelstein B, Kinzler KW. Association of the APC tumor suppressor protein with catenins. Science. 1993 Dec 10;262(5140):1734–7.

59. Groden J, Thliveris A, Samowitz W, Carlson M, Gelbert L, Albertsen H, et al. Identification and characterization of the familial adenomatous polyposis coli gene. Cell. 1991 Aug 9;66(3):589–600.

60. Klaus A, Birchmeier W. Wnt signalling and its impact on development and cancer. Nat Rev Cancer. 2008 May;8(5):387–98.

61. Thomas KR, Capecchi MR. Targeted disruption of the murine int-1 proto-oncogene resulting in severe abnormalities in midbrain and cerebellar development. Nature. 1990 Aug 30;346(6287):847–50.

62. Gibson P, Tong Y, Robinson G, Thompson MC, Currle DS, Eden C, et al. Subtypes of medulloblastoma have distinct developmental origins. Nature. 2010 Dec 23;468(7327):1095–9.

63. Gilbertson RJ, Ellison DW. The origins of medulloblastoma subtypes. Annu Rev Pathol. 2008;3:341–65.

64. Baeza N, Masuoka J, Kleihues P, Ohgaki H. AXIN1 mutations but not deletions in cerebellar medulloblastomas. Oncogene. 2003 Jan 30;22(4):632–6.

65. Eberhart CG, Kepner JL, Goldthwaite PT, Kun LE, Duffner PK, Friedman HS, et al. Histopathologic grading of medulloblastomas: a Pediatric Oncology Group study. Cancer. 2002 Jan 15;94(2):552–60.

66. Cho Y-J, Tsherniak A, Tamayo P, Santagata S, Ligon A, Greulich H, et al. Integrative genomic analysis of medulloblastoma identifies a molecular subgroup that drives poor clinical outcome. J Clin Oncol Off J Am Soc Clin Oncol. 2011 Apr 10;29(11):1424–30.

67. Fiaschetti G, Schroeder C, Castelletti D, Arcaro A, Westermann F, Baumgartner M, et al. NOTCH ligands JAG1 and JAG2 as critical pro-survival factors in childhood medulloblastoma. Acta Neuropathol Commun. 2014;2:39.

68. Perry A, Brat DJ. Practical Surgical Neuropathology: A Diagnostic Approach: A Volume in the Pattern Recognition series [Internet]. Elsevier Health Sciences; 2010 [cited 2015 Oct 21]. Available from:

https://books.google.co.in/books?hl=en&lr=&id=FqdwaTagUlsC&oi=fnd&pg=PP1&dq=arie+perry+textbook+neuropathology&ots=YWhUv92L77&sig=6ARdPMLum68lSTIjKSpicyDGpfQ

69. Kool M, Koster J, Bunt J, Hasselt NE, Lakeman A, van Sluis P, et al. Integrated genomics identifies five medulloblastoma subtypes with distinct genetic profiles, pathway signatures and clinicopathological features. PloS One. 2008;3(8):e3088.

70. McManamy CS, Pears J, Weston CL, Hanzely Z, Ironside JW, Taylor RE, et al. Nodule formation and desmoplasia in medulloblastomas-defining the nodular/desmoplastic variant and its biological behavior. Brain Pathol Zurich Switz. 2007 Apr;17(2):151–64.

71. Louis DN, Ohgaki H, Wiestler OD, Cavenee WK, Burger PC, Jouvet A, et al. The 2007 WHO classification of tumours of the central nervous system. Acta Neuropathol (Berl). 2007 Aug;114(2):97–109.

72. Ellison D. Classifying the medulloblastoma: insights from morphology and molecular genetics. Neuropathol Appl Neurobiol. 2002 Aug;28(4):257–82.

73. Eberhart CG, Kepner JL, Goldthwaite PT, Kun LE, Duffner PK, Friedman HS, et al. Histopathologic grading of medulloblastomas: a Pediatric Oncology Group study. Cancer. 2002 Jan 15;94(2):552–60.

74. Helton KJ, Fouladi M, Boop FA, Perry A, Dalton J, Kun L, et al. Medullomyoblastoma: a radiographic and clinicopathologic analysis of six cases and review of the literature. Cancer. 2004 Sep 15;101(6):1445–54.

75. Höll T, Kleihues P, Yasargil MG, Wiestler OD. Cerebellar medullomyoblastoma with advanced neuronal differentiation and hamartomatous component. Acta Neuropathol (Berl). 1991;82(5):408–13.

76. Kubota KC, Itoh T, Yamada Y, Yamaguchi S, Ishida Y, Nakasu Y, et al. Melanocytic medulloblastoma with ganglioneurocytomatous differentiation: a case report. Neuropathol Off J Jpn Soc Neuropathol. 2009 Feb;29(1):72–7.

77. Burger PC, Grahmann FC, Bliestle A, Kleihues P. Differentiation in the medulloblastoma. A histological and immunohistochemical study. Acta Neuropathol (Berl). 1987;73(2):115–23.

78. Packer RJ, Gajjar A, Vezina G, Rorke-Adams L, Burger PC, Robertson PL, et al. Phase III study of craniospinal radiation therapy followed by adjuvant chemotherapy for newly diagnosed average-risk medulloblastoma. J Clin Oncol Off J Am Soc Clin Oncol. 2006 Sep 1;24(25):4202–8.

79. Parsons DW, Li M, Zhang X, Jones S, Leary RJ, Lin JC-H, et al. The genetic landscape of the childhood cancer medulloblastoma. Science. 2011 Jan 28;331(6016):435–9.

80. Jakacki RI. Treatment strategies for high-risk medulloblastoma and supratentorial primitive neuroectodermal tumors. Review of the literature. J Neurosurg. 2005 Jan;102(1 Suppl):44–52.

81. Pomeroy SL, Tamayo P, Gaasenbeek M, Sturla LM, Angelo M, McLaughlin ME, et al. Prediction of central nervous system embryonal tumour outcome based on gene expression. Nature. 2002 Jan 24;415(6870):436–42.

82. Gottardo NG, Gajjar A. Current therapy for medulloblastoma. Curr Treat Options Neurol. 2006 Jul;8(4):319–34.

83. Thompson MC, Fuller C, Hogg TL, Dalton J, Finkelstein D, Lau CC, et al. Genomics identifies medulloblastoma subgroups that are enriched for specific genetic alterations. J Clin Oncol Off J Am Soc Clin Oncol. 2006 Apr 20;24(12):1924–31.

84. Cho Y-J, Tsherniak A, Tamayo P, Santagata S, Ligon A, Greulich H, et al. Integrative genomic analysis of medulloblastoma identifies a molecular subgroup that drives poor clinical outcome. J Clin Oncol Off J Am Soc Clin Oncol. 2011 Apr 10;29(11):1424–30.

85. Taylor MD, Northcott PA, Korshunov A, Remke M, Cho Y-J, Clifford SC, et al. Molecular subgroups of medulloblastoma: the current consensus. Acta Neuropathol (Berl). 2012 Apr;123(4):465–72.

86. Turcot J, Despres JP, St Pierre F. Malignant tumors of the central nervous system associated with familial polyposis of the colon: report of two cases. Dis Colon Rectum. 1959 Oct;2:465–8.

87. Gupta T, Shirsat N, Jalali R. Molecular Subgrouping of Medulloblastoma: Impact Upon Research and Clinical Practice. Curr Pediatr Rev. 2015;11(2):106–19.

88. Goodrich LV, Milenković L, Higgins KM, Scott MP. Altered neural cell fates and medulloblastoma in mouse patched mutants. Science. 1997 Aug 22;277(5329):1109–13.

89. Northcott PA, Hielscher T, Dubuc A, Mack S, Shih D, Remke M, et al. Pediatric and adult sonic hedgehog medulloblastomas are clinically and molecularly distinct. Acta Neuropathol (Berl). 2011 Aug;122(2):231–40.

90. DeSouza R-M, Jones BRT, Lowis SP, Kurian KM. Pediatric medulloblastoma - update on molecular classification driving targeted therapies. Front Oncol. 2014;4:176.

91. Packer RJ, Rood BR, MacDonald TJ. Medulloblastoma: present concepts of stratification into risk groups. Pediatr Neurosurg. 2003 Jul;39(2):60–7.

92. Bartlett F, Kortmann R, Saran F. Medulloblastoma. Clin Oncol R Coll Radiol G B. 2013 Jan;25(1):36–45.

93. Pizer BL, Clifford SC. The potential impact of tumour biology on improved clinical practice for medulloblastoma: progress towards biologically driven clinical trials. Br J Neurosurg. 2009 Aug;23(4):364–75.

94. Ellison DW, Kocak M, Dalton J, Megahed H, Lusher ME, Ryan SL, et al. Definition of disease-risk stratification groups in childhood medulloblastoma using combined clinical, pathologic, and molecular variables. J Clin Oncol Off J Am Soc Clin Oncol. 2011 Apr 10;29(11):1400–7.

95. Fossati P, Ricardi U, Orecchia R. Pediatric medulloblastoma: toxicity of current treatment and potential role of protontherapy. Cancer Treat Rev. 2009 Feb;35(1):79–96.

96. Bien-Willner GA, López-Terrada D, Bhattacharjee MB, Patel KU, Stankiewicz P, Lupski JR, et al. Early recurrence in standard-risk medulloblastoma patients with the common idic(17)(p11.2) rearrangement. Neuro-Oncol. 2012 Jul;14(7):831–40.

97. Gottardo NG, Gajjar A. Current therapy for medulloblastoma. Curr Treat Options Neurol. 2006 Jul;8(4):319–34.

98. Carlotti CG, Smith C, Rutka JT. The molecular genetics of medulloblastoma: an assessment of new therapeutic targets. Neurosurg Rev. 2008 Oct;31(4):359–68; discussion 368–9.

99. Smoll NR, Drummond KJ. The incidence of medulloblastomas and primitive neurectodermal tumours in adults and children. J Clin Neurosci Off J Neurosurg Soc Australas. 2012 Nov;19(11):1541–4.

100. Millard NE, Braganca KCD. Medulloblastoma. J Child Neurol. 2015 Sep 2;0883073815600866.

101. Giordana MT, Schiffer P, Lanotte M, Girardi P, Chio A. Epidemiology of adult medulloblastoma. Int J Cancer J Int Cancer. 1999 Mar 1;80(5):689–92.

102. Massimino M, Antonelli M, Gandola L, Miceli R, Pollo B, Biassoni V, et al. Histological variants of medulloblastoma are the most powerful clinical prognostic indicators. Pediatr Blood Cancer. 2013 Feb;60(2):210–6.

103. Giangaspero F, Perilongo G, Fondelli MP, Brisigotti M, Carollo C, Burnelli R, et al. Medulloblastoma with extensive nodularity: a variant with favorable prognosis. J Neurosurg. 1999 Dec 1;91(6):971–7.

104. Garrè ML, Cama A, Bagnasco F, Morana G, Giangaspero F, Brisigotti M, et al. Medulloblastoma variants: age-dependent occurrence and relation to Gorlin syndrome--a new clinical perspective. Clin Cancer Res Off J Am Assoc Cancer Res. 2009 Apr 1;15(7):2463–71.

105. Kaur K, Kakkar A, Kumar A, Mallick S, Julka PK, Gupta D, et al. Integrating Molecular Subclassification of Medulloblastomas into Routine Clinical Practice: A Simplified Approach. Brain Pathol Zurich Switz. 2015 Jul 29;

106. Grammel D, Warmuth-Metz M, von Bueren AO, Kool M, Pietsch T, Kretzschmar HA, et al. Sonic hedgehog-associated medulloblastoma arising from the cochlear nuclei of the brainstem. Acta Neuropathol (Berl). 2012 Apr;123(4):601–14.

107. Gupta T, Shirsat N, Jalali R. Molecular Subgrouping of Medulloblastoma: Impact Upon Research and Clinical Practice. Curr Pediatr Rev. 2015;11(2):106–19.

108. Taylor MD, Mainprize TG, Rutka JT, Becker L, Bayani J, Drake JM. Medulloblastoma in a child with Rubenstein-Taybi Syndrome: case report and review of the literature. Pediatr Neurosurg. 2001 Nov;35(5):235–8.

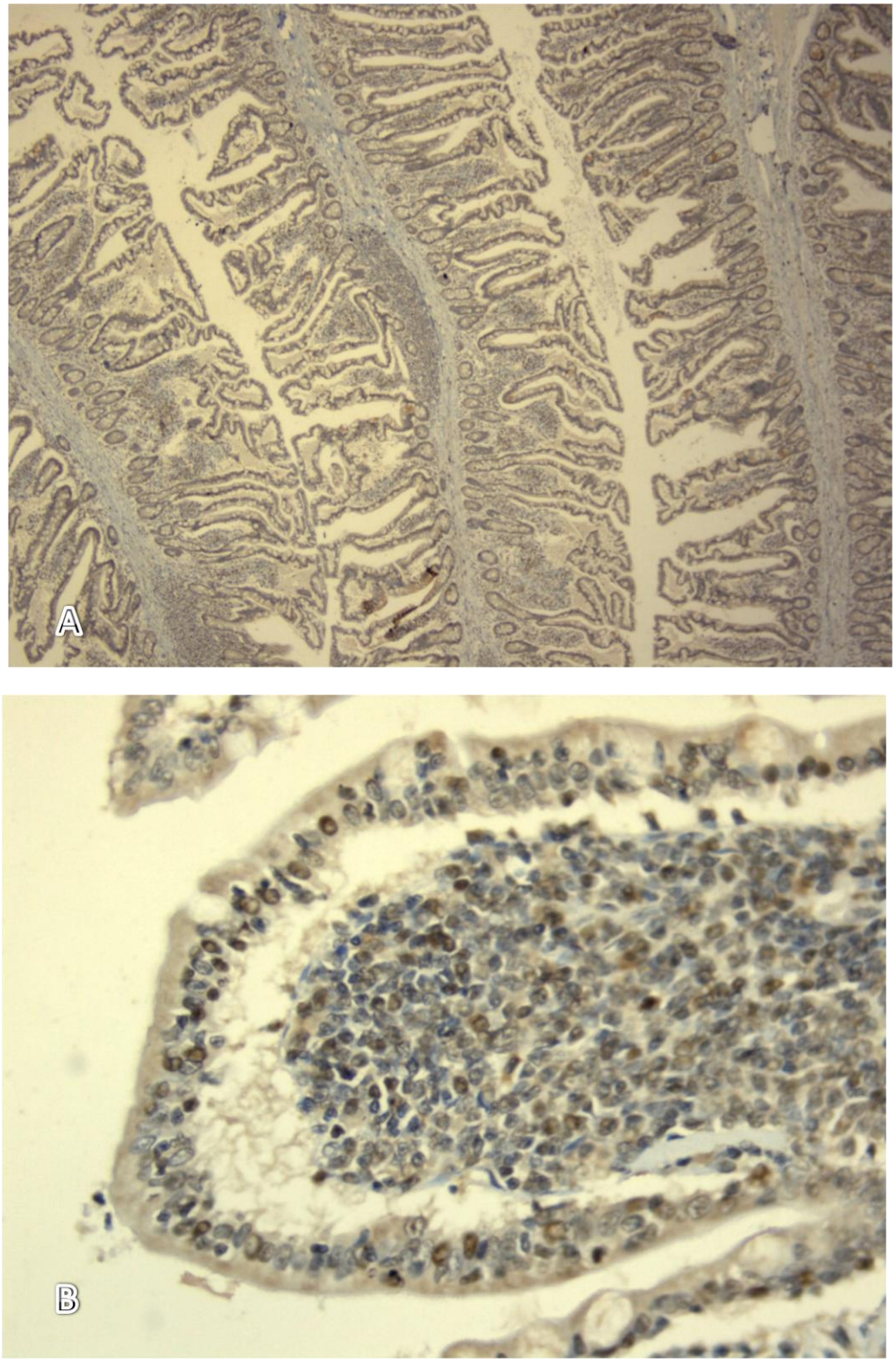


Figure 1(a)&(b): β- catenin: Nuclear positivity in control colonic epithelium. A)Low power magnification(x100), B)High power magnification(x400)

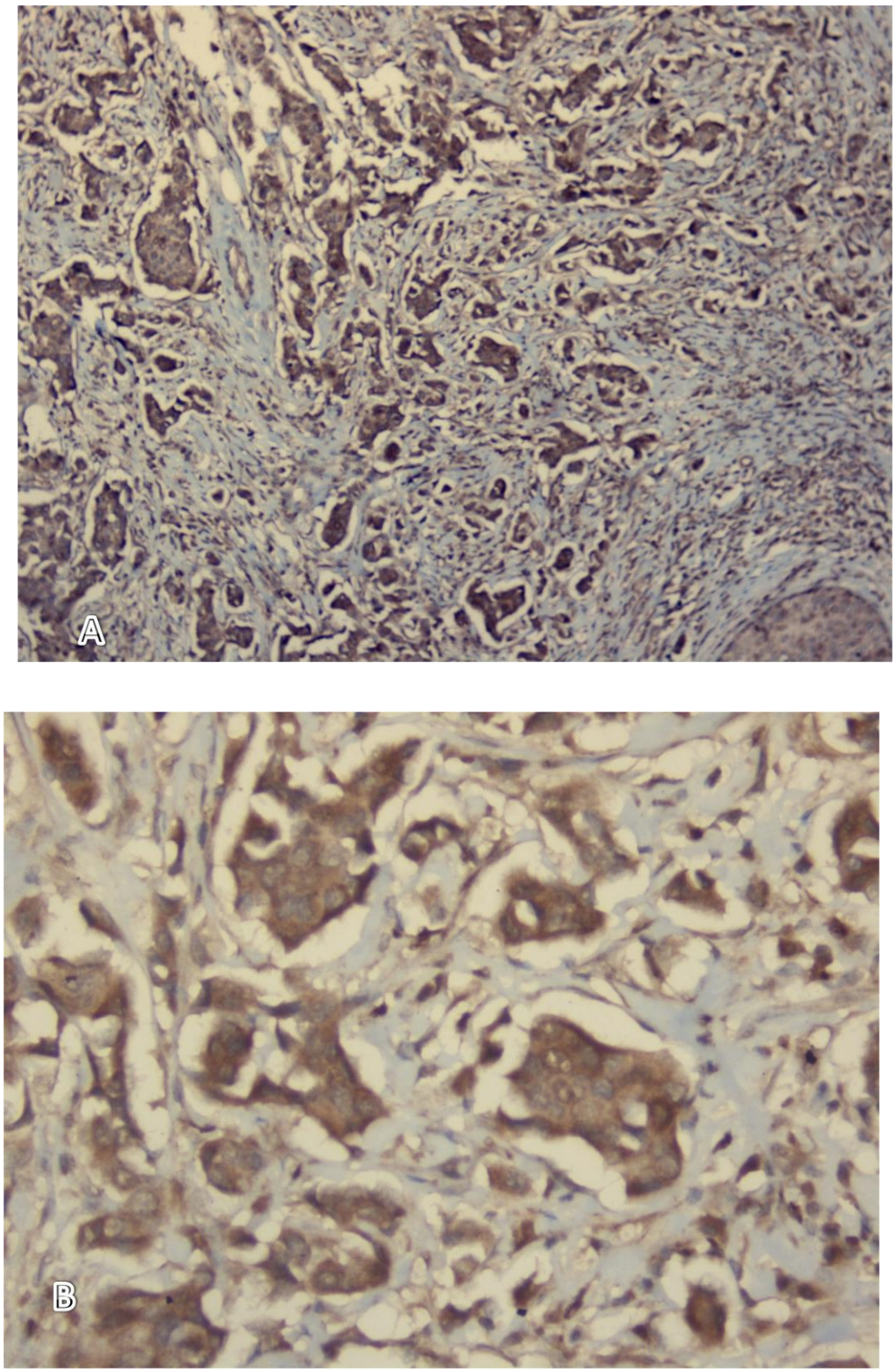


Figure 2(a)& (b): GAB-1: Showing cytoplasmic positivity in control invasive ductal carcinoma, breast A)Low power magnification (x100), B)High power magnification (x400)

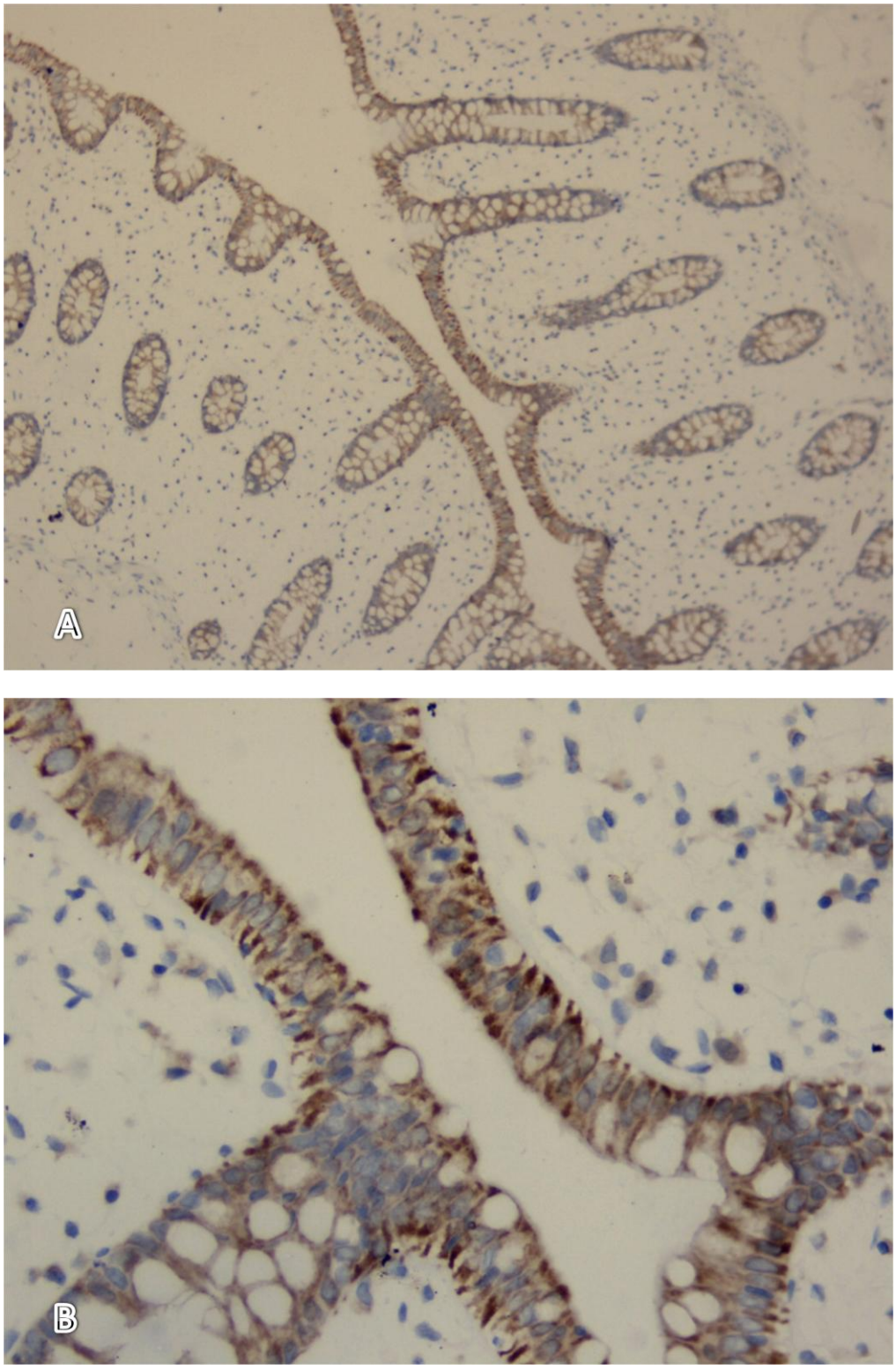


Figure 3(a)&3(b): NPR-3: Cytoplasmic positivity in control normal colon. A) Low Magnification (x100), B) High power magnification (x400)

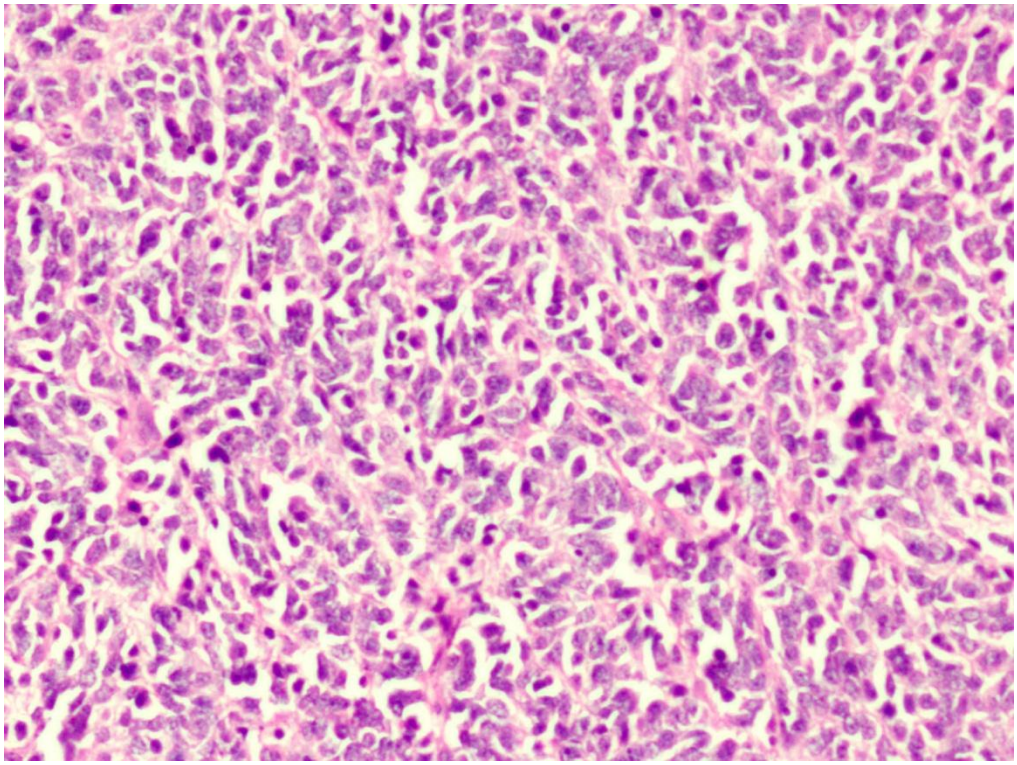


Figure 19(a):Classic variant of medulloblastoma with closely packed cells (H&E, x100)

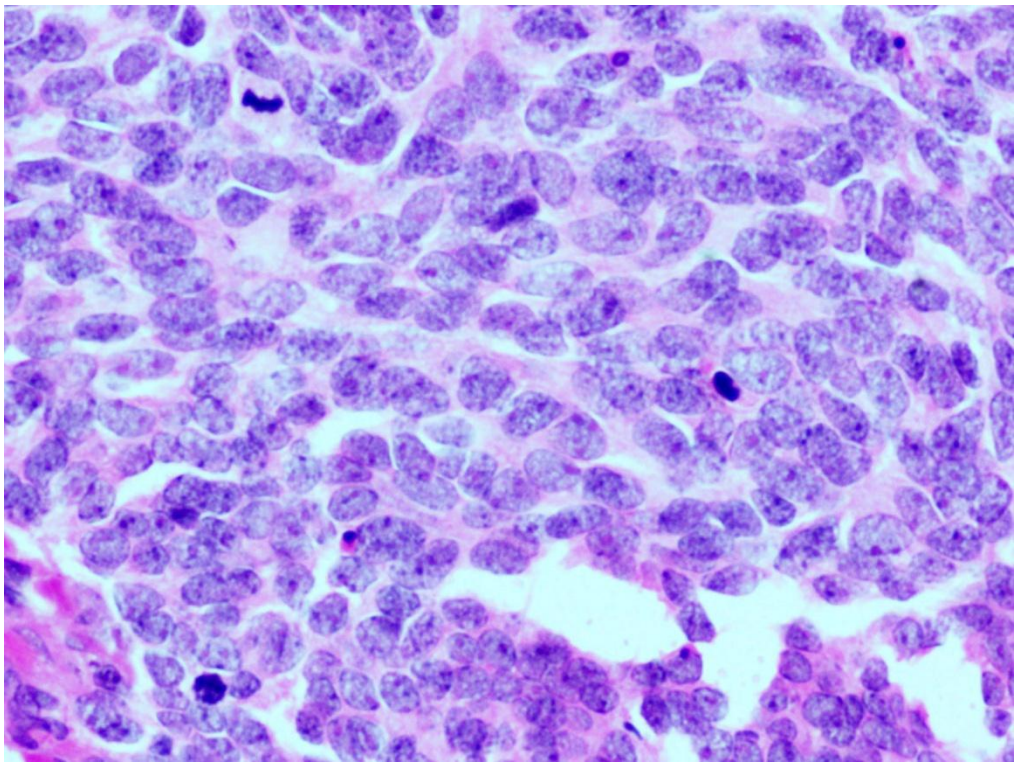


Figure 19(b): Classic variant of medulloblastoma with medium sized hyperchromatic nuclei and scant cytoplasm (H&E, x400)

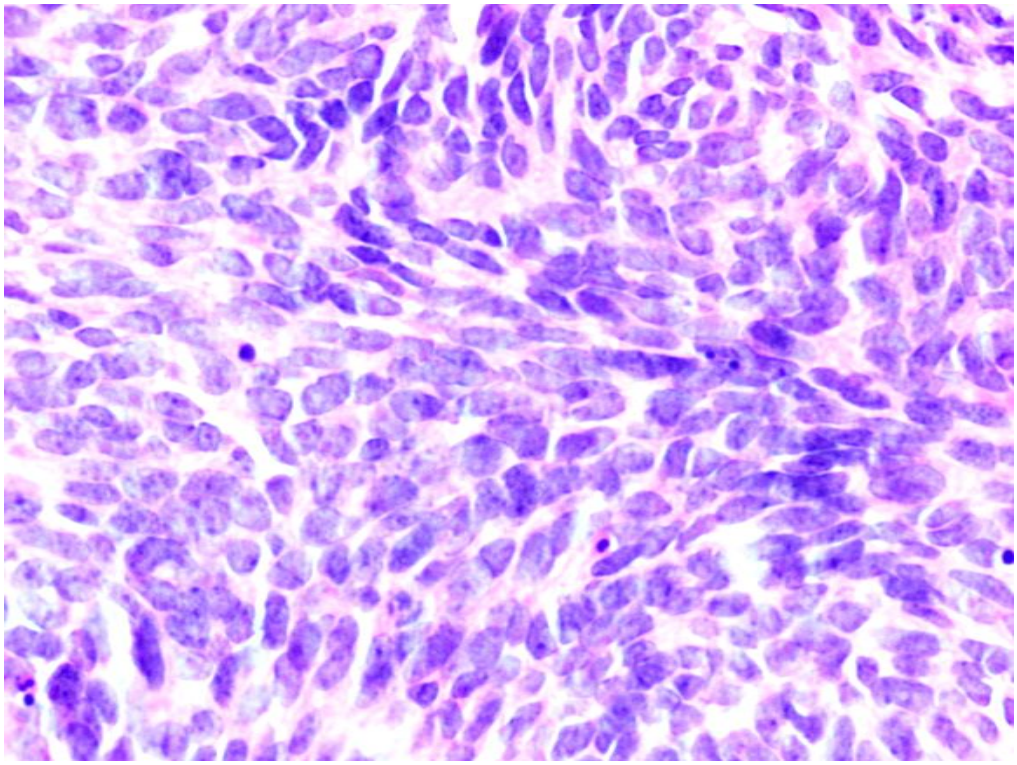


Figure 19(c): Carrot shaped nuclei in classic medulloblastoma (H&E, x400)

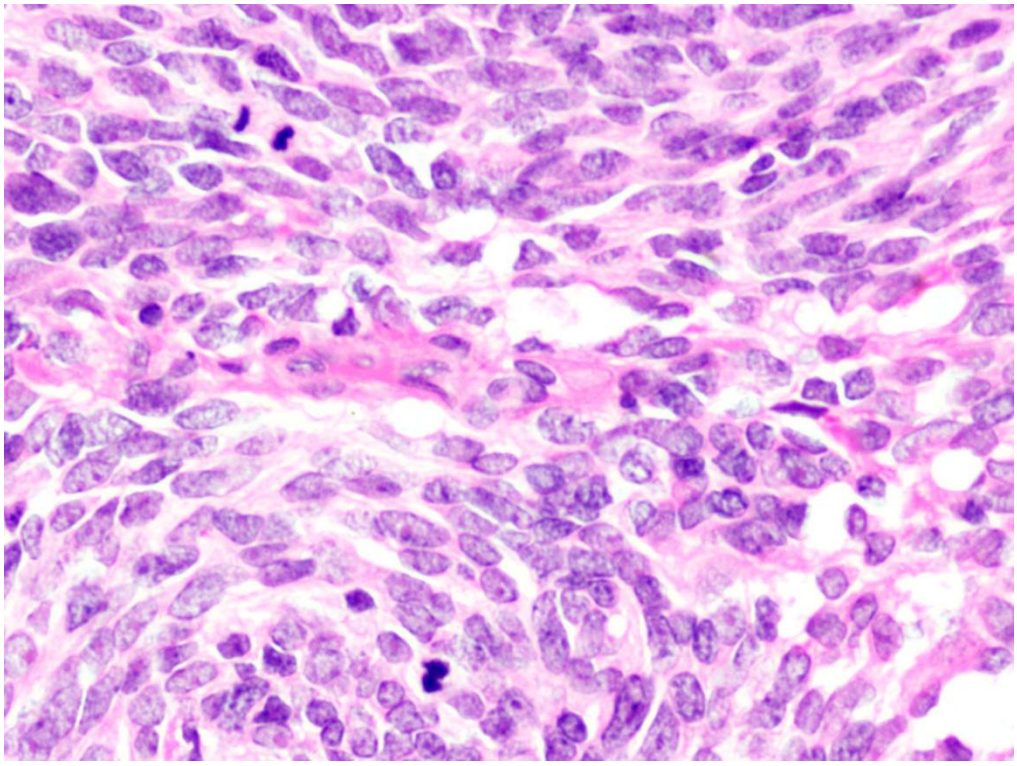


Figure 19(d):Numerous mitotic figures in classic variant of medulloblastoma (H&E, x400)

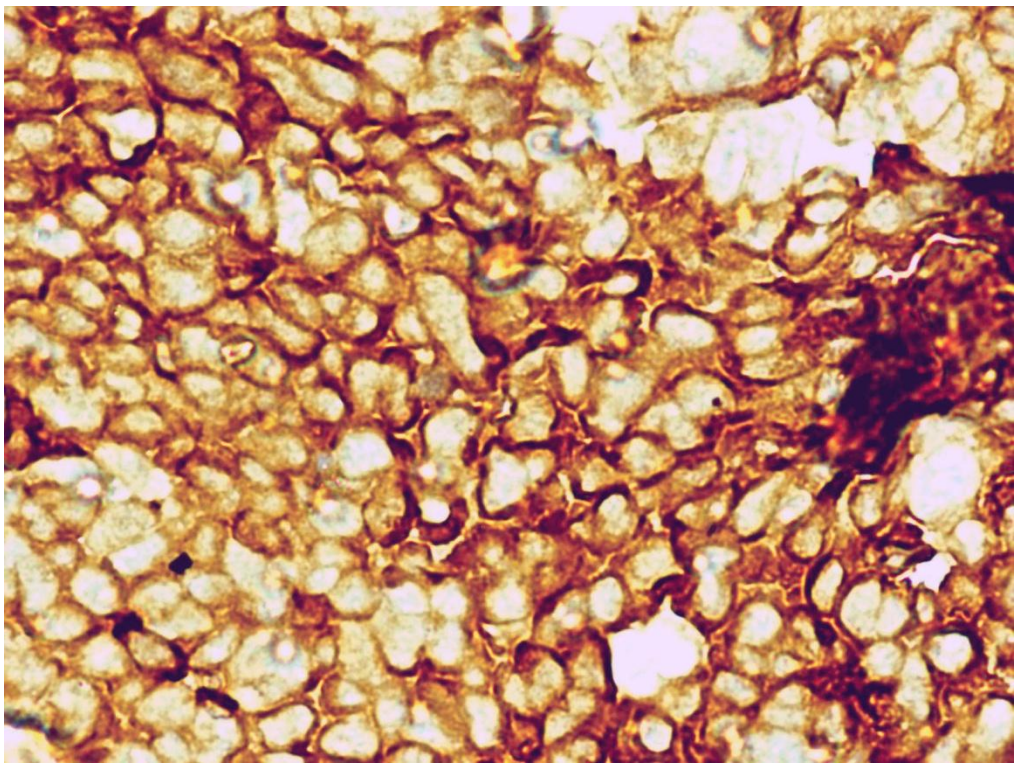


Figure 19(e):Synaptophysin positivity in classic variant of medulloblastoma (x400)

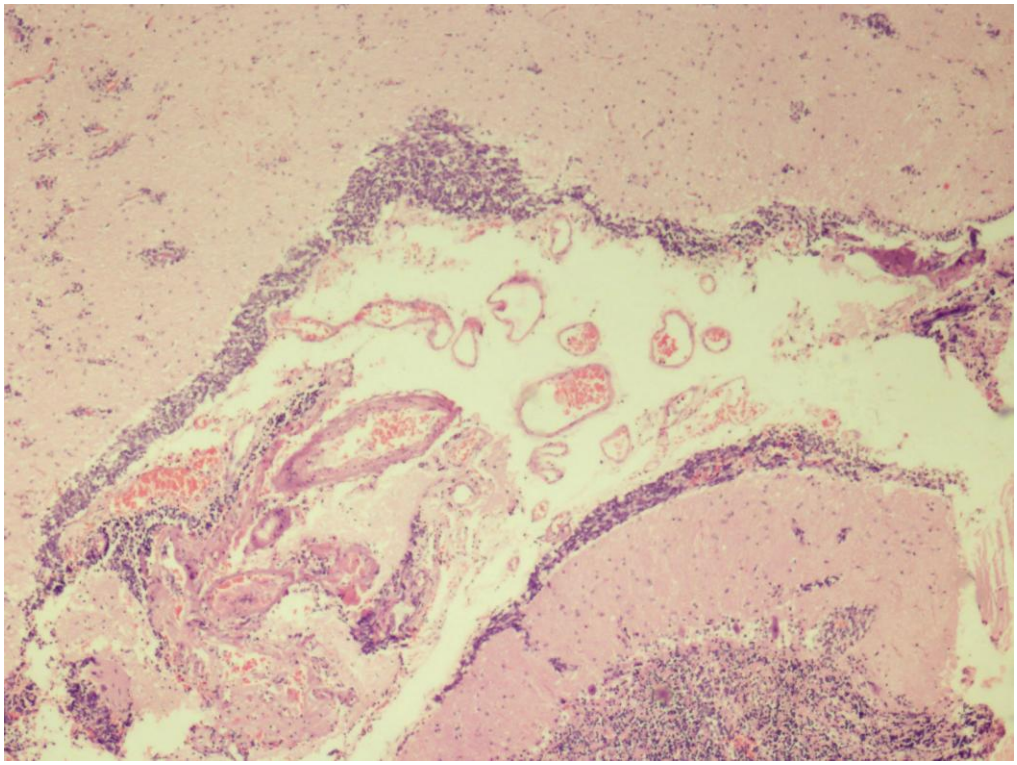


Figure 19(f): Classic medulloblastoma with leptomeningeal invasion (H&E, x40)

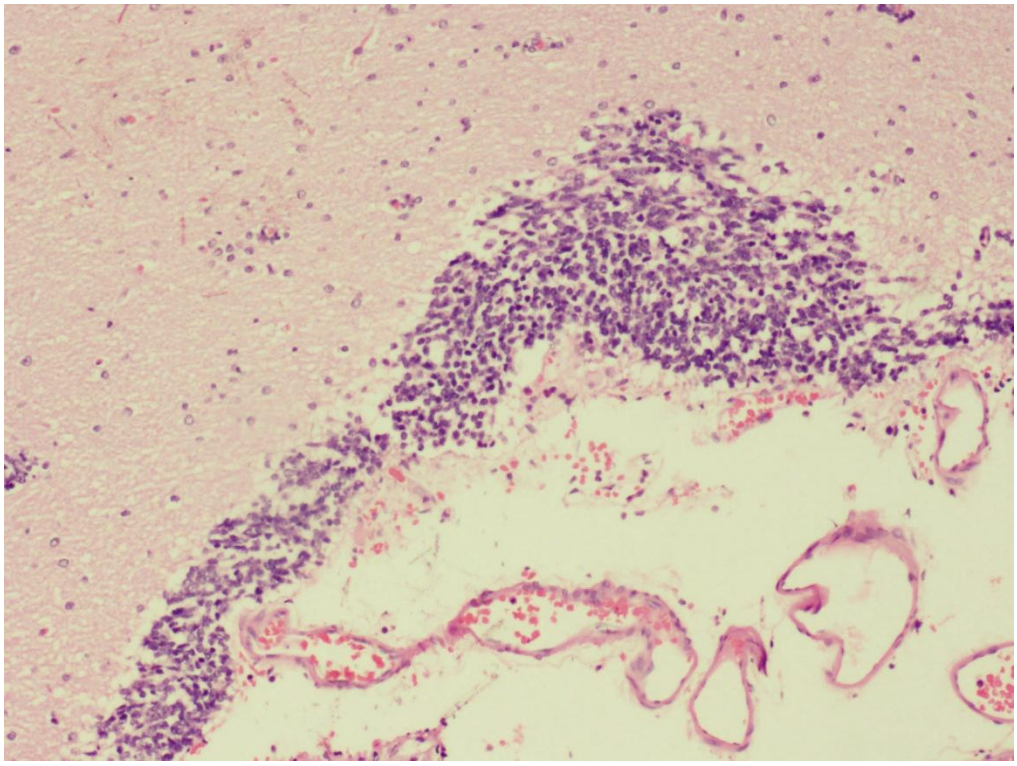


Figure 19(g): Classic medulloblastoma with leptomeningeal invasion (H&E, x100)

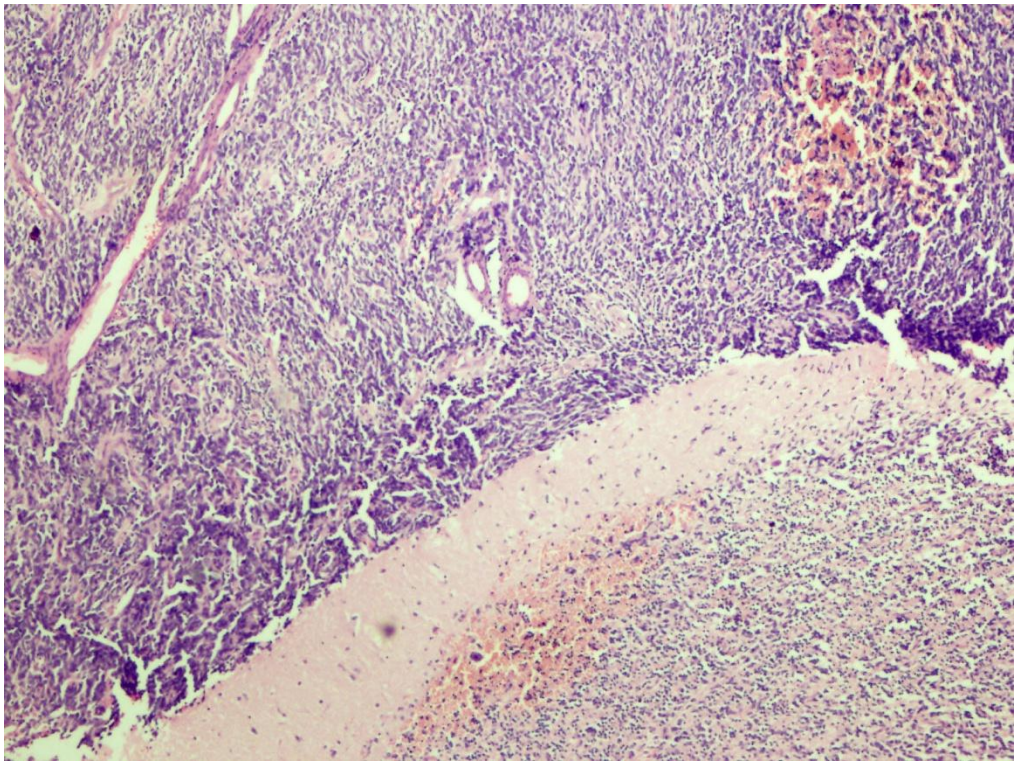


Figure 19(h): Classic medulloblastoma with leptomeningeal invasion (H&E, x200)

Figure 19(i): Stromal desmoplasia associated with leptomeningeal invasion (Retic,x200)

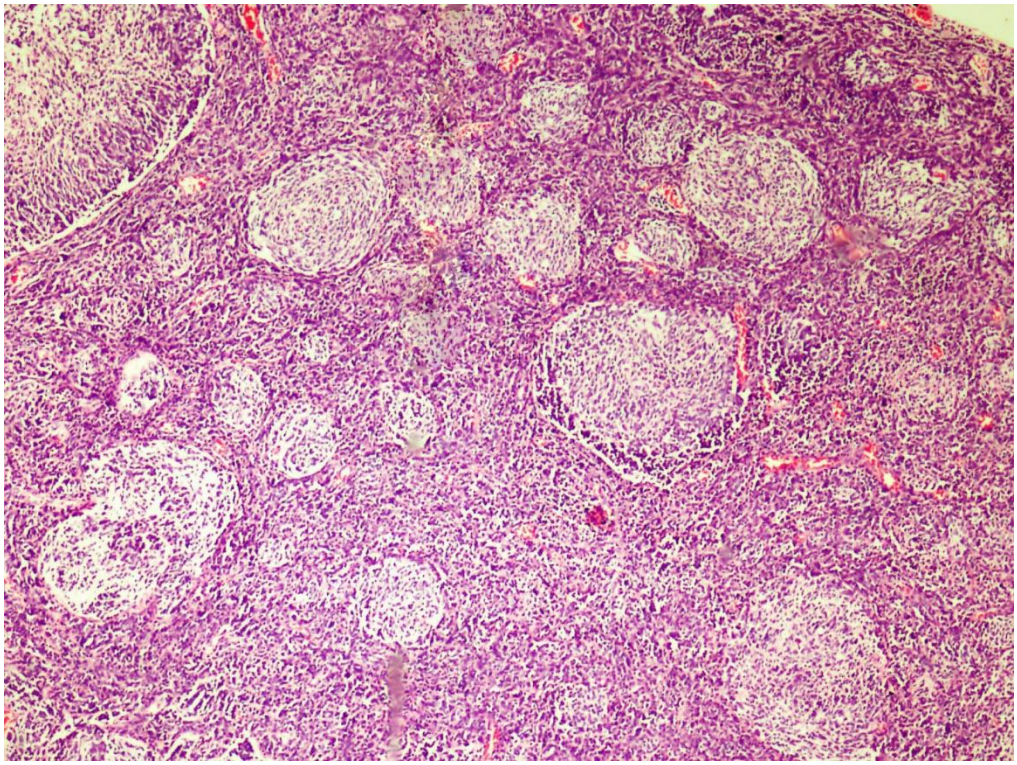


Figure 20(a):Desmoplastic/nodular medulloblastoma highlighting the pale nodules(H&E,x100)

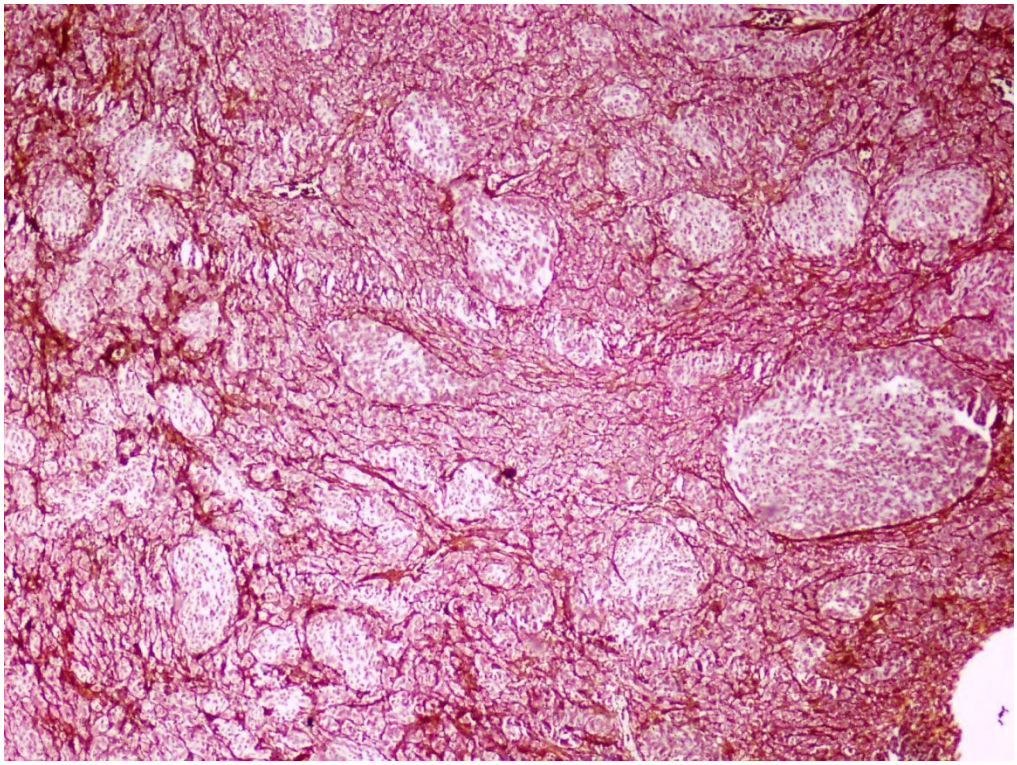
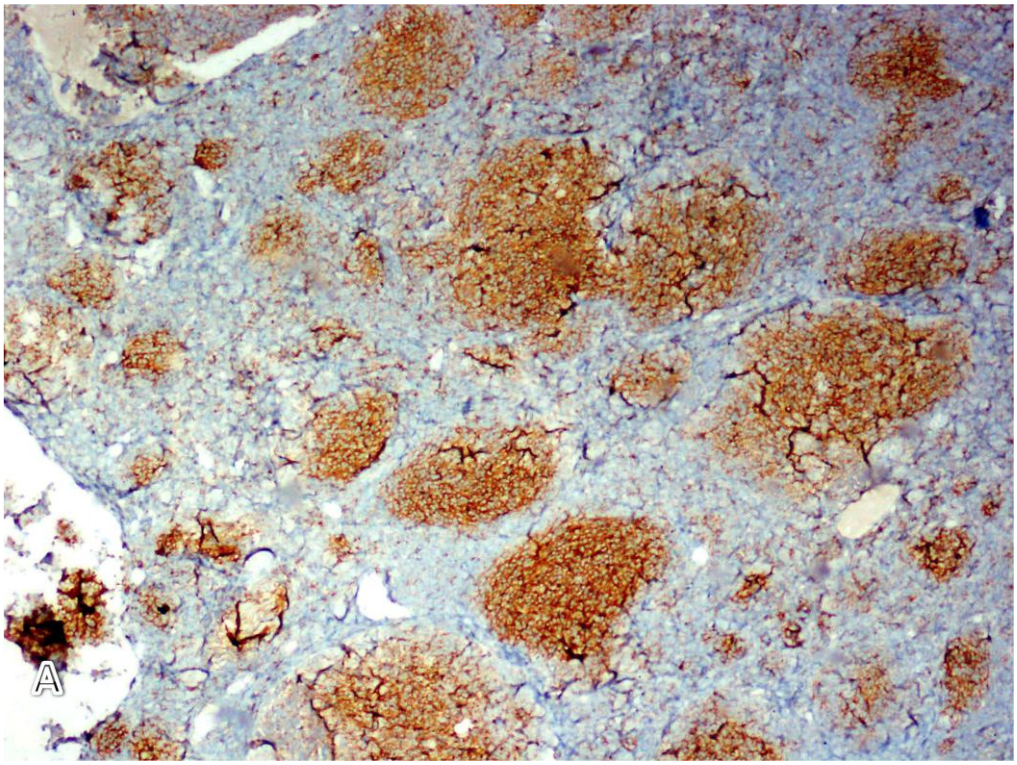


Figure 20(b): Nodular reticulin free zones in a desmoplastic medulloblastoma (Retic,x100)



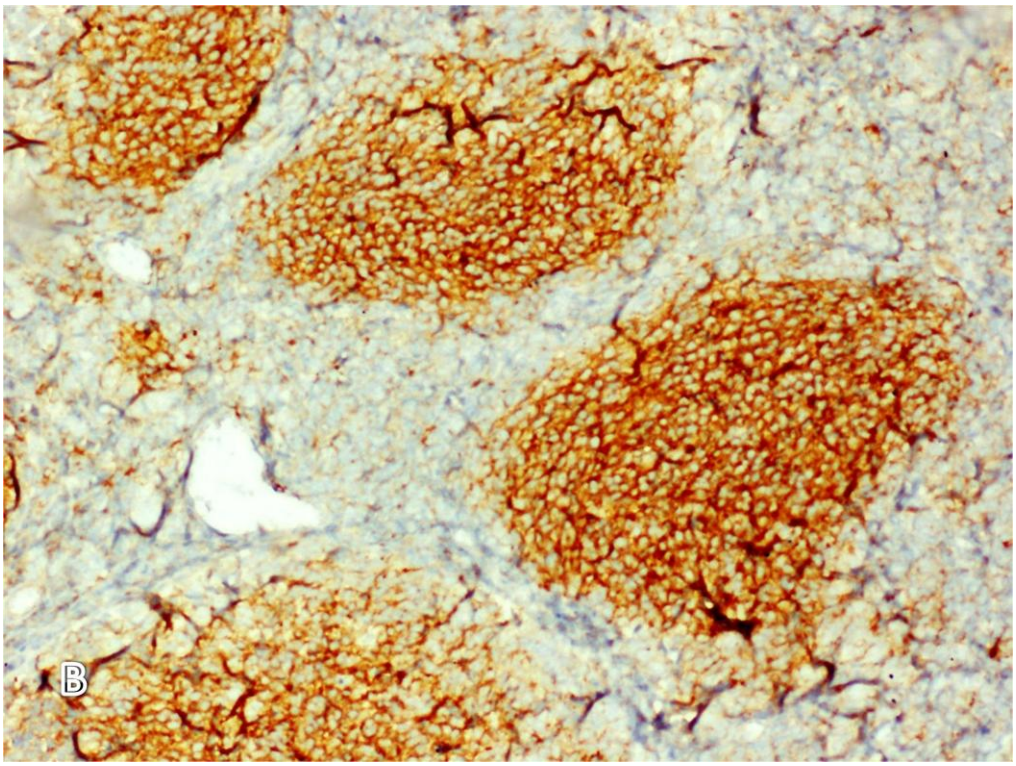


Figure 20(c): Neuronal differentiation in the nodules evidenced by immunopositivity for synaptophysin. A)Low power magnification(x40) B)High power magnification(x200)

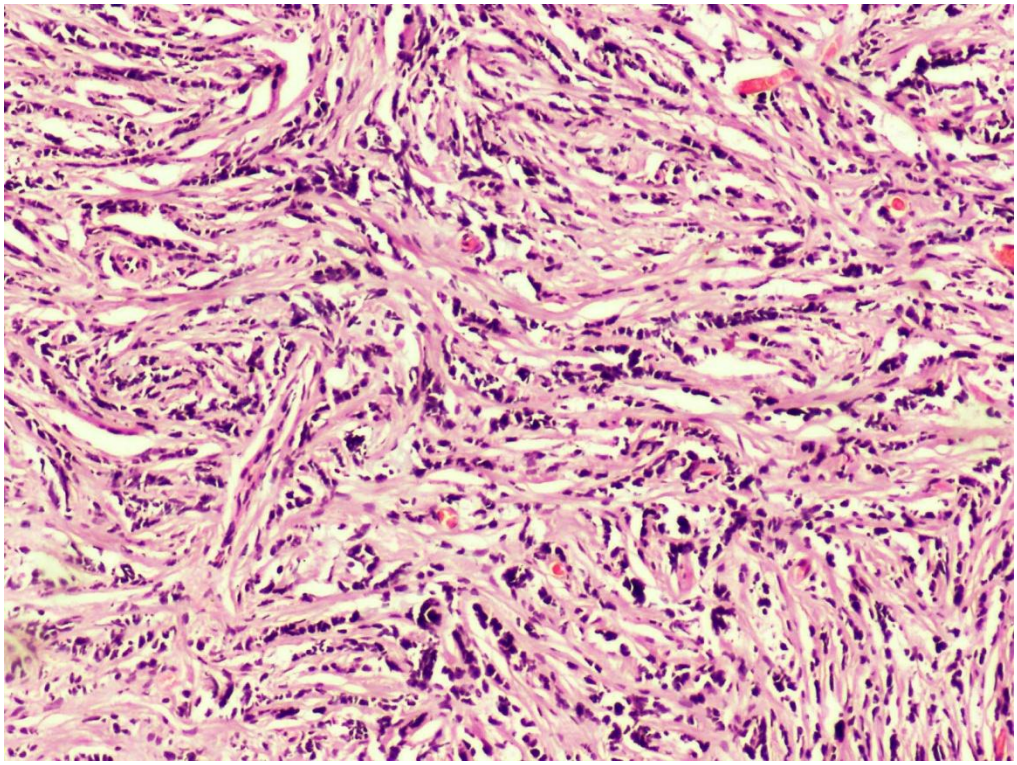


Figure 20(d):Streaming in a classic medulloblastoma with absent nodularity (H&E, x100)

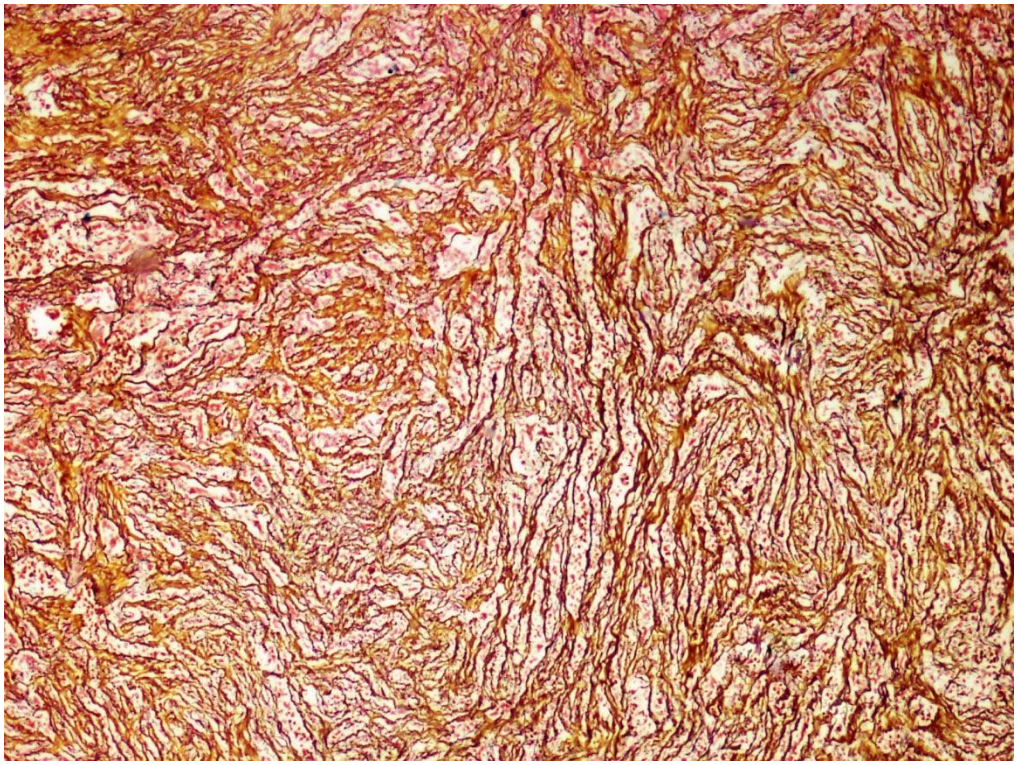


Figure 20(e): Associated stromal desmoplasia. Note the absence of reticulin free nodules. (Retic, x100)

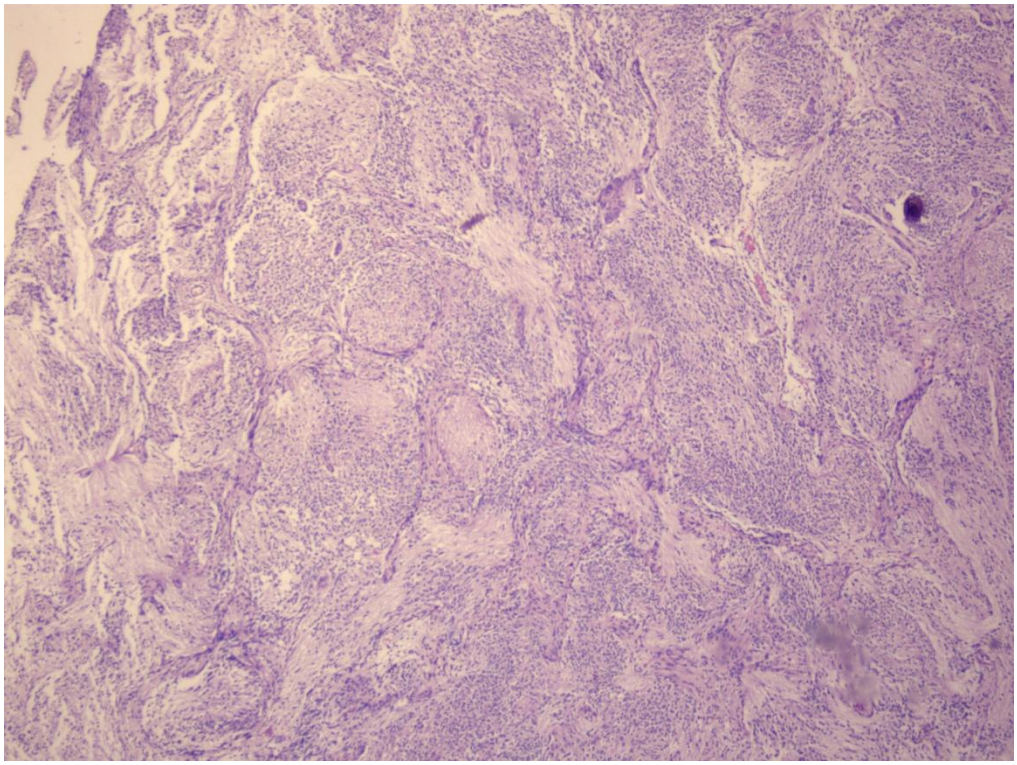


Figure 21(a) :Medulloblastoma with extensive nodularity (H&E, x40)

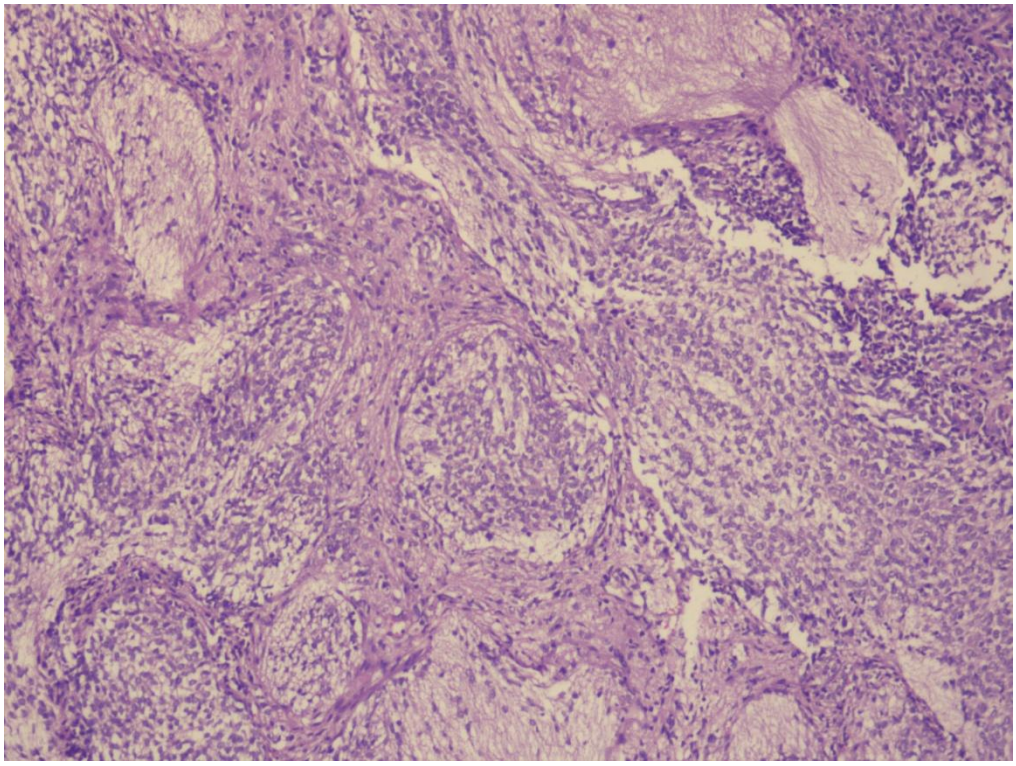


Figure 21(b): Medulloblastoma with extensive nodularity (H&E, x100)

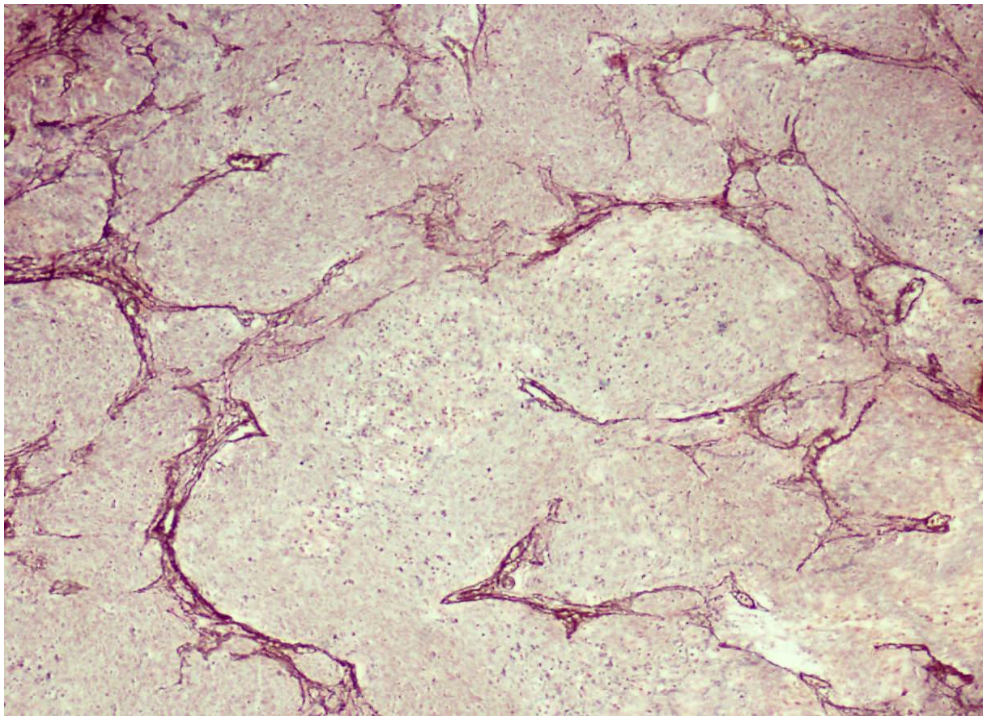


Figure 21(c): Medulloblastoma with extensive nodularity with large reticulin free zones(Retic, x100)

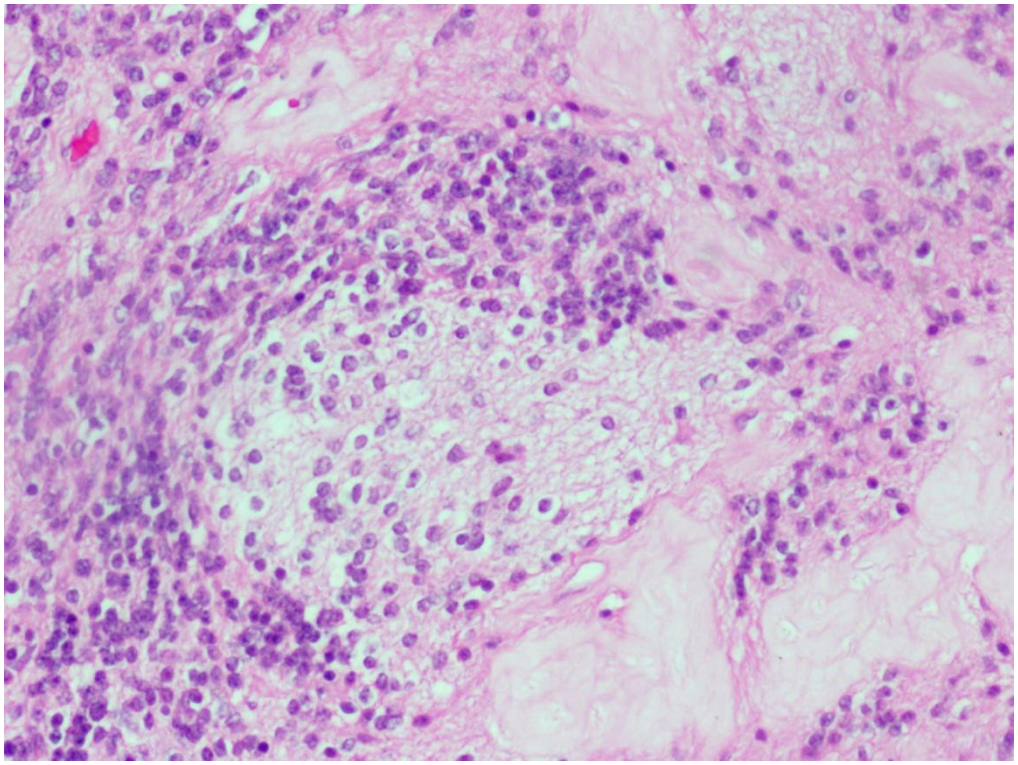


Figure 21(d): Medulloblastoma with extensive nodularity with neurocytic differentiation(H&E, x200)

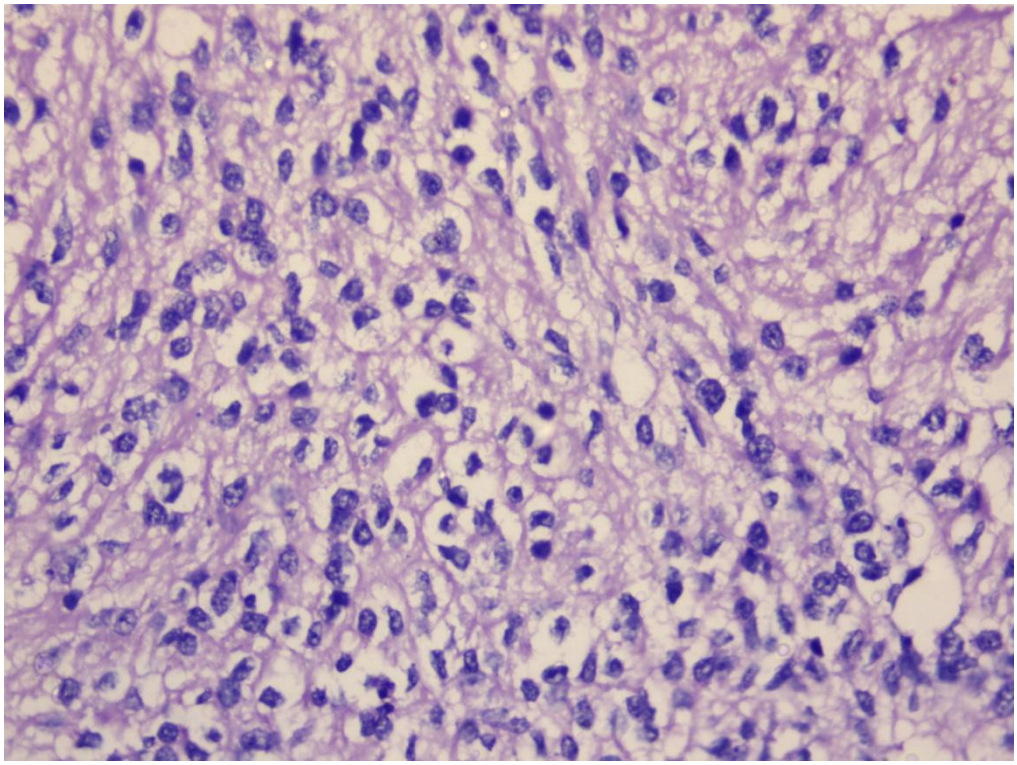


Figure 21(e): Medulloblastoma with extensive nodularity with neurocytic differentiation (H&E, x400)

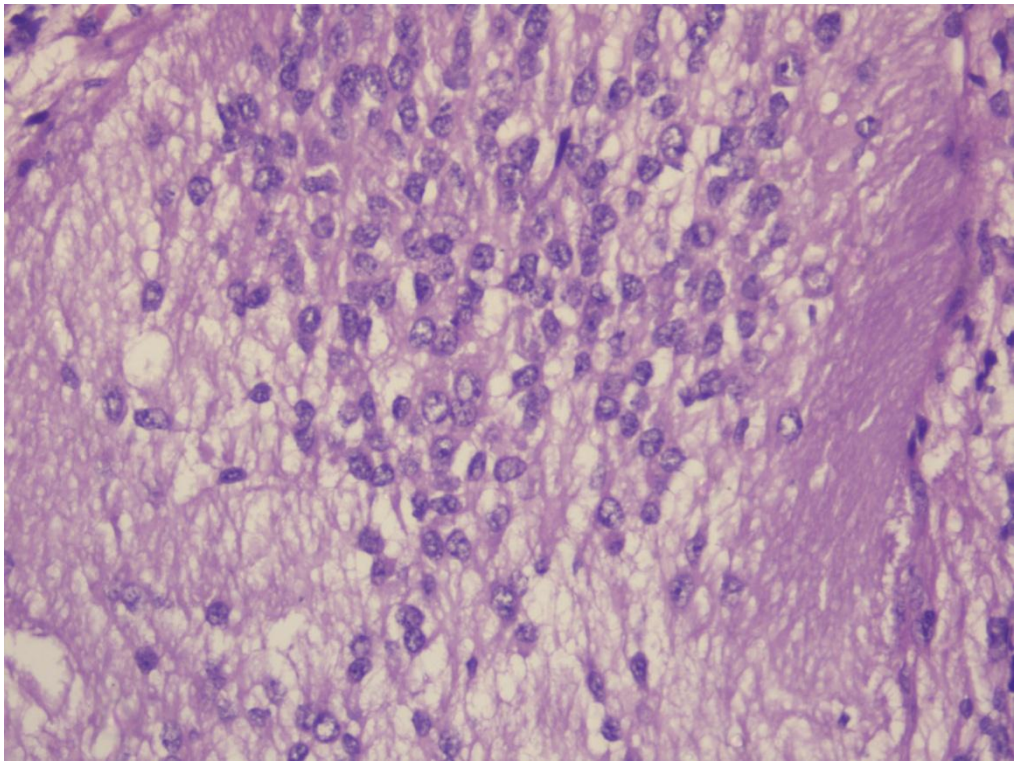


Figure 21(f): Medulloblastoma with extensive nodularity with small round neurocytic cells in a fibrillary background(H&E, x400)

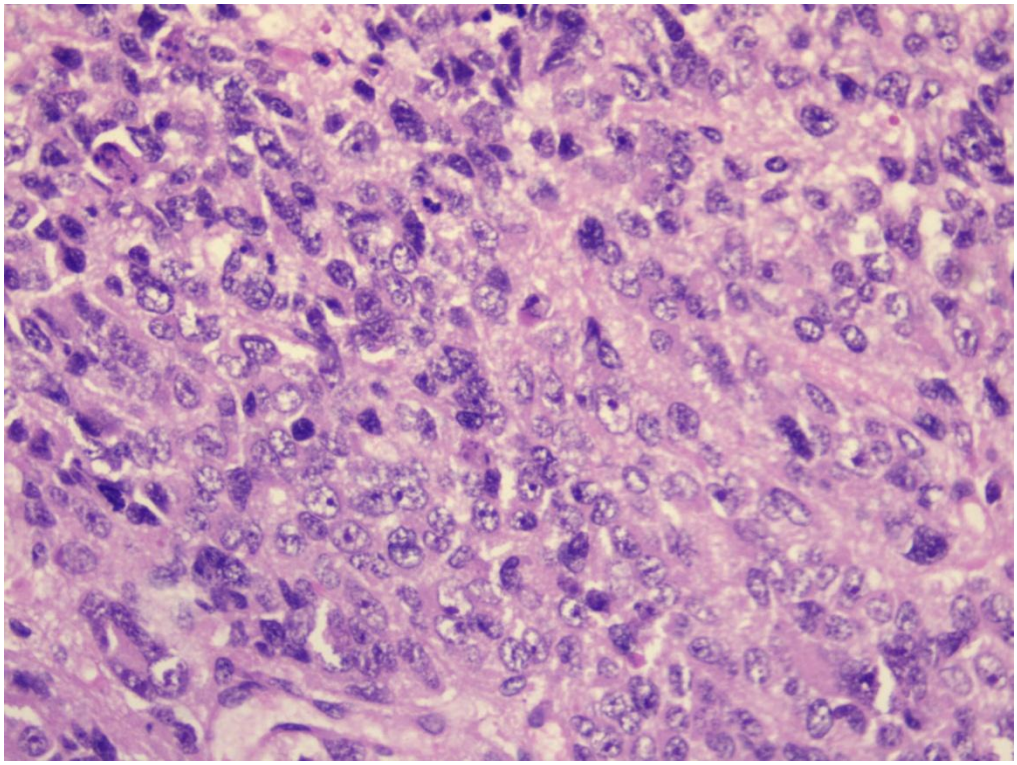


Figure 22(a): Large cell medulloblastoma large vesicular nuclei with prominent nucleoli (H&E,x400)

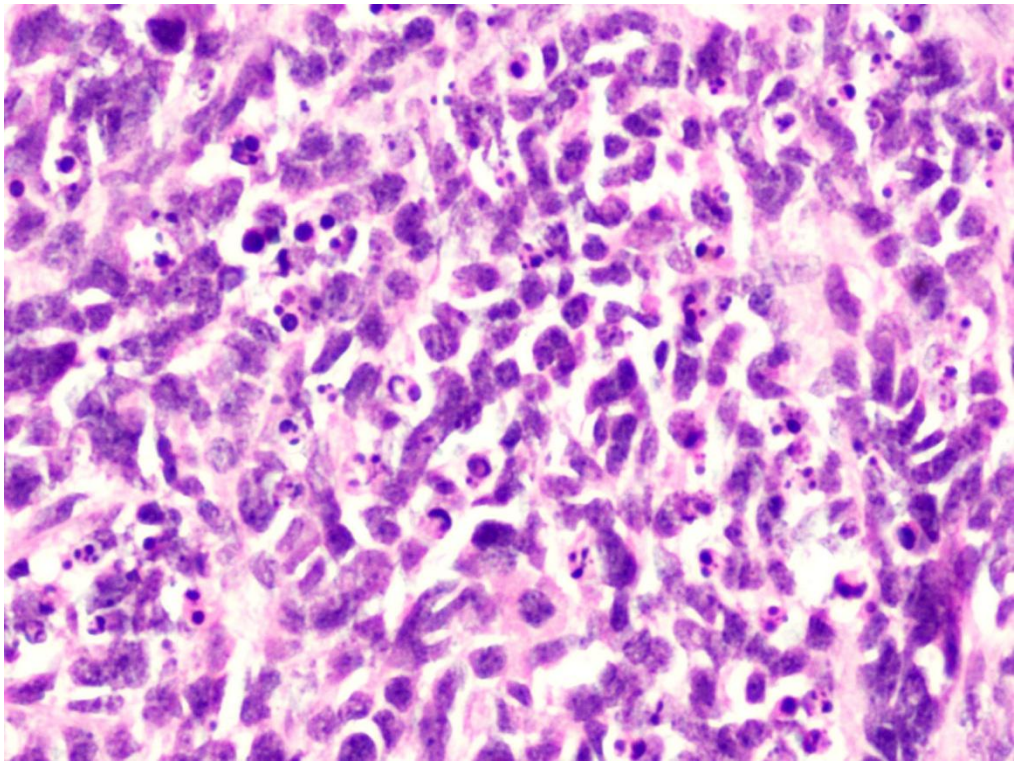


Figure 22(b):Numerous apoptotic bodies in a large cell medulloblastoma(H&E, x400)

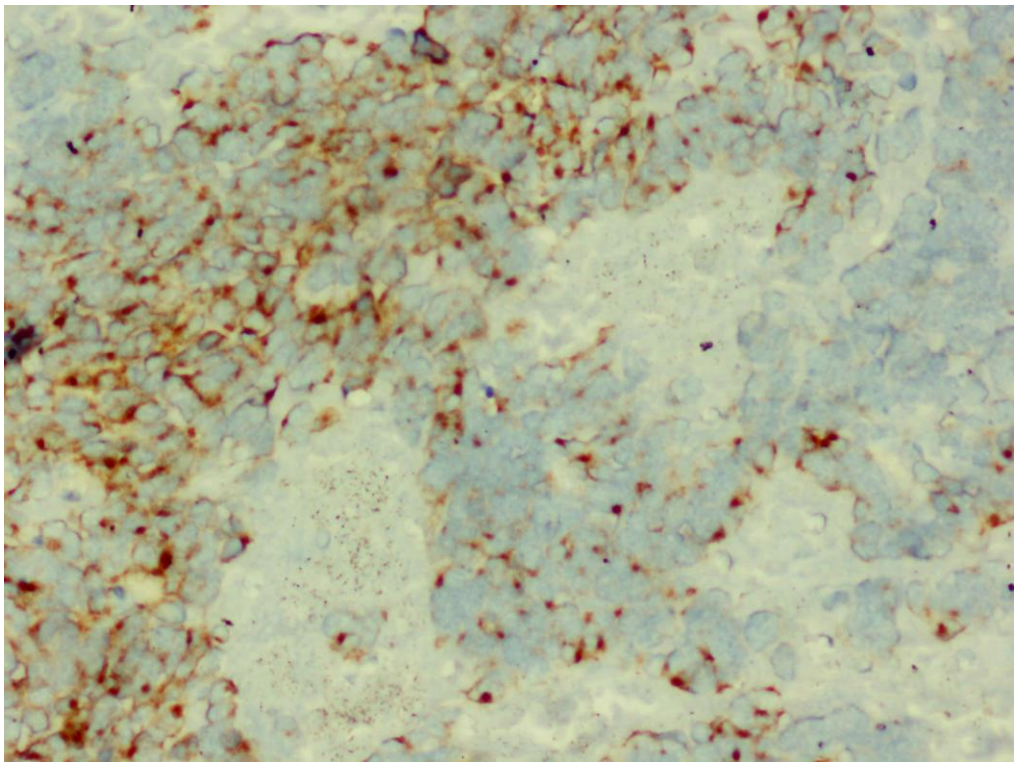


Figure 22(c):Synaptophysin displaying dot like positivity in a large cell medulloblastoma(x400)

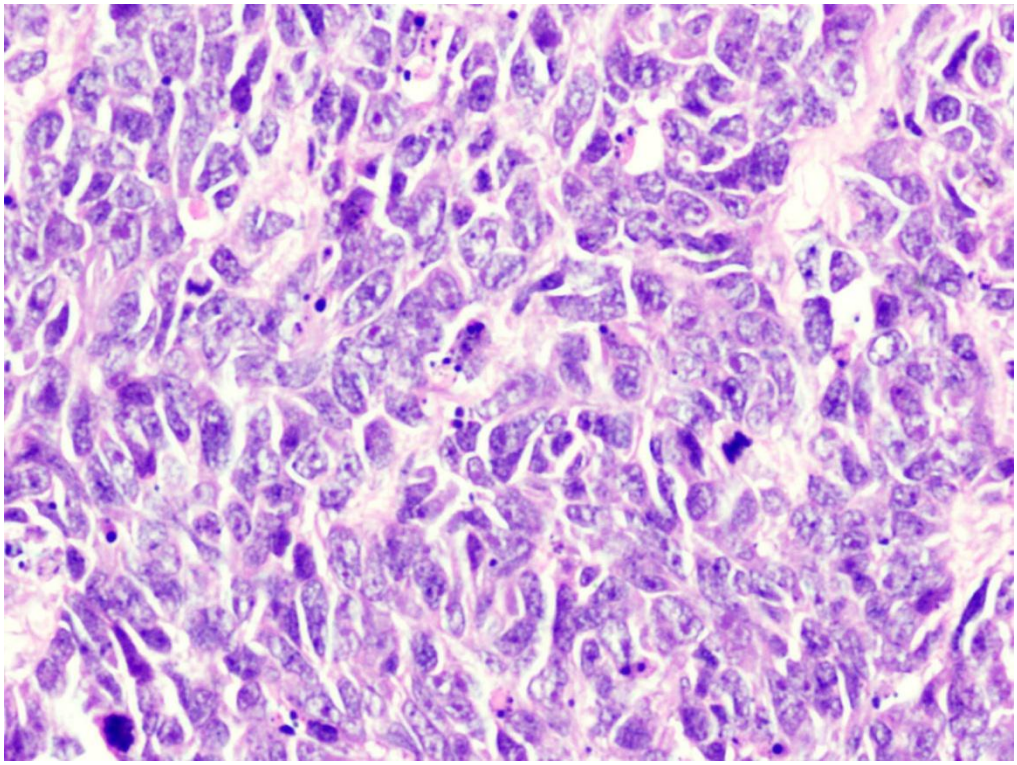


Figure 23(a):Anaplastic medulloblastoma with increased nuclear pleomorphism and high mitotic activity(H&E,x400)

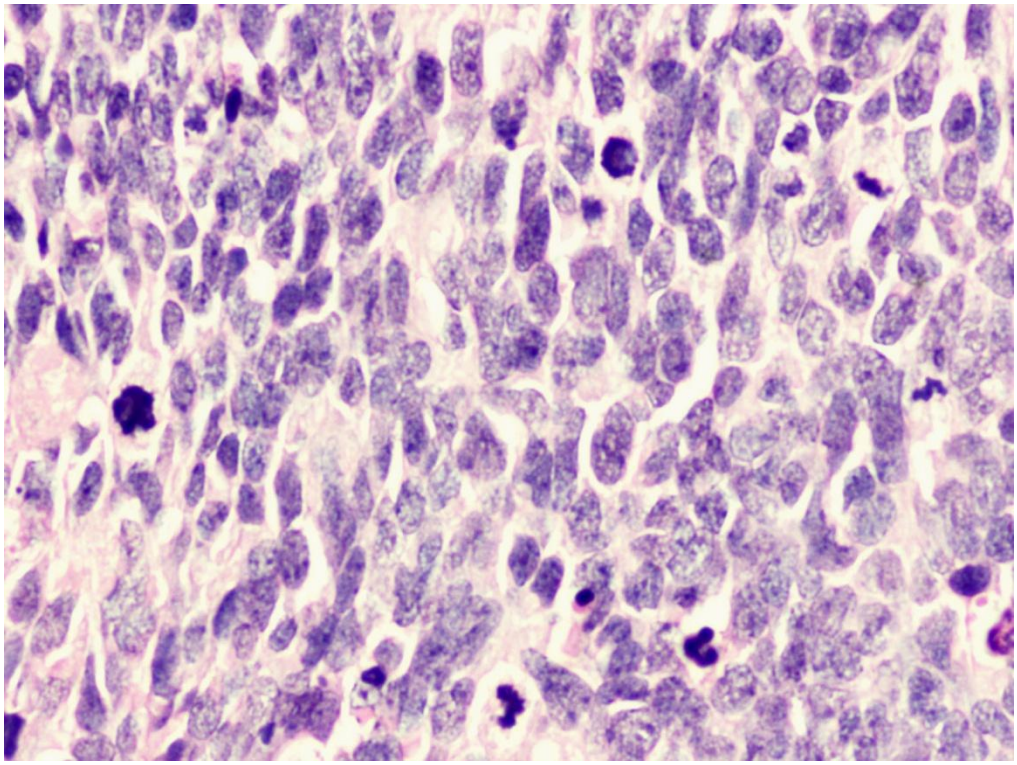


Figure 23(b): Brisk mitotic activity in an anaplastic medulloblastoma(H&E, x400)

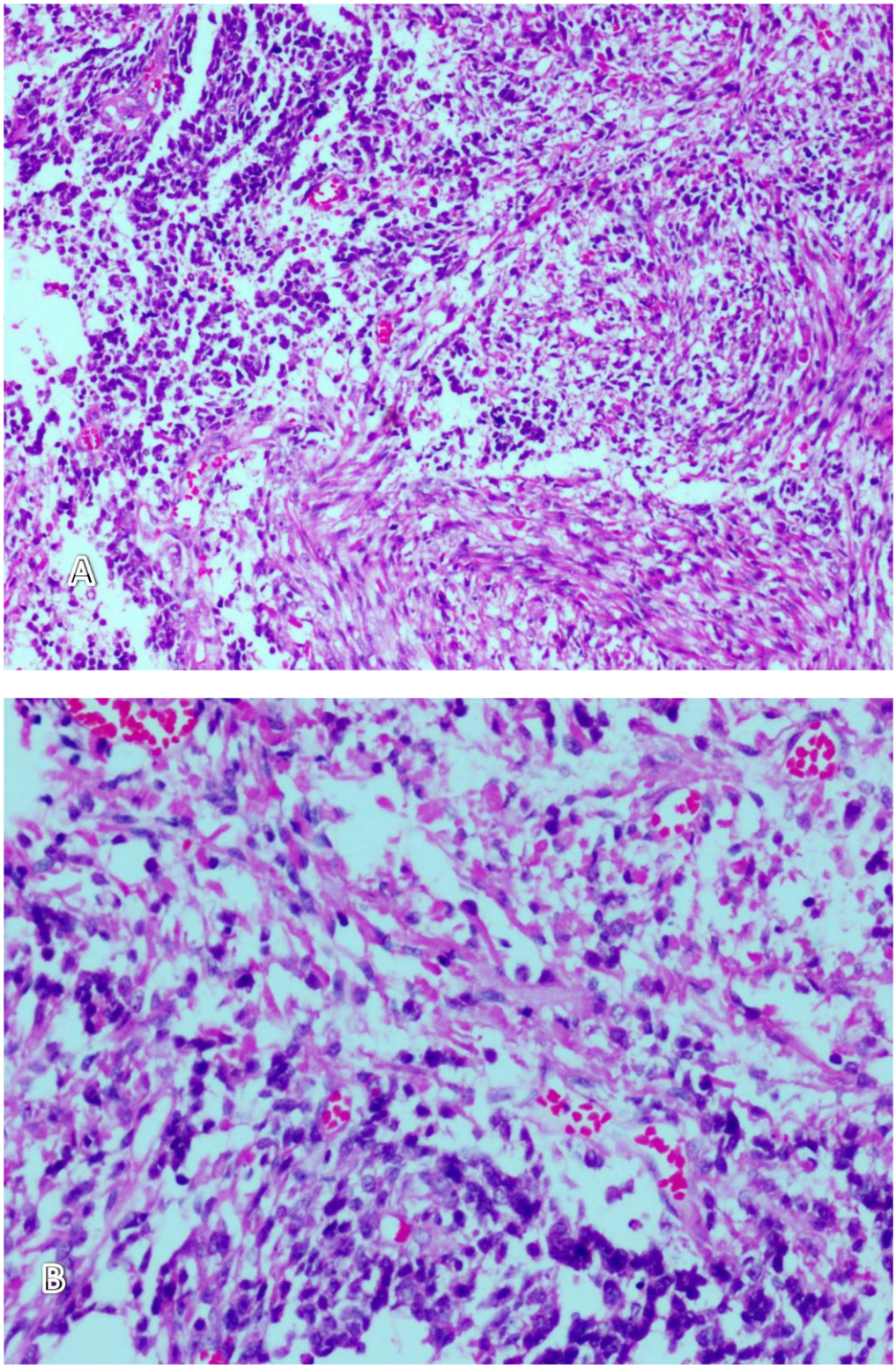


Figure 24(a&b): Medulloblastoma with myogenic differentiation . Note the component of classic varaint in thelower left quadrant. A)Low power magnification (H&E,x100) B)High power magnification (H&E,x200)

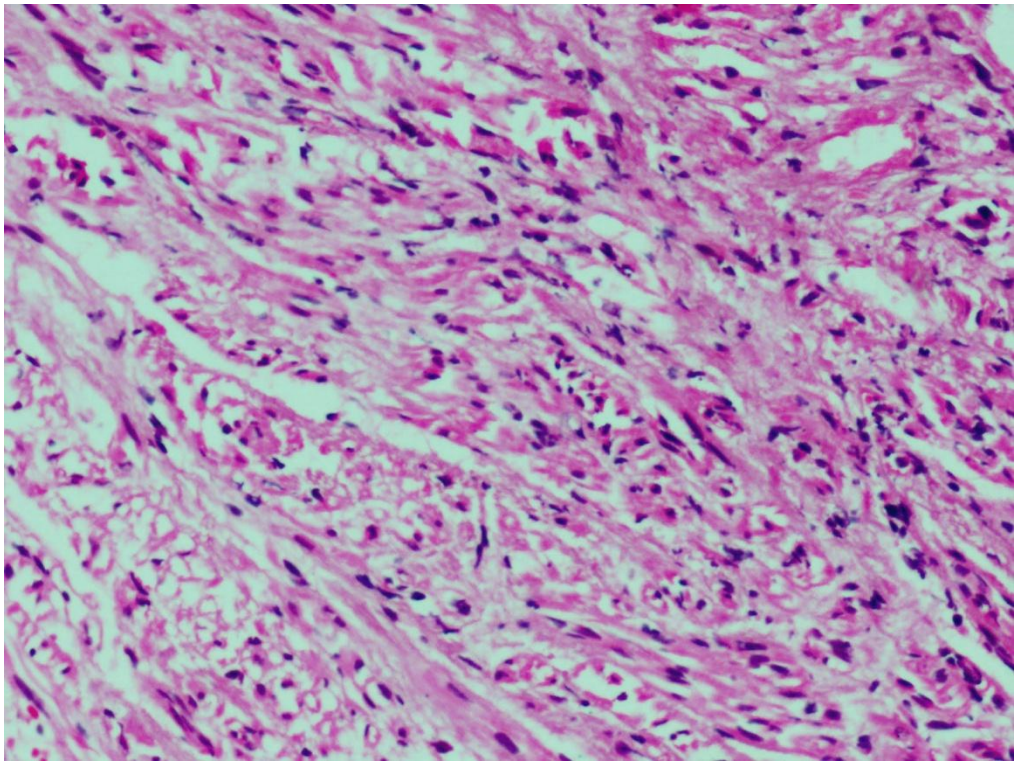


Figure 24(c) :Medulloblastoma with myogenic differentiation highlighting the strap cell (H&E, x200)

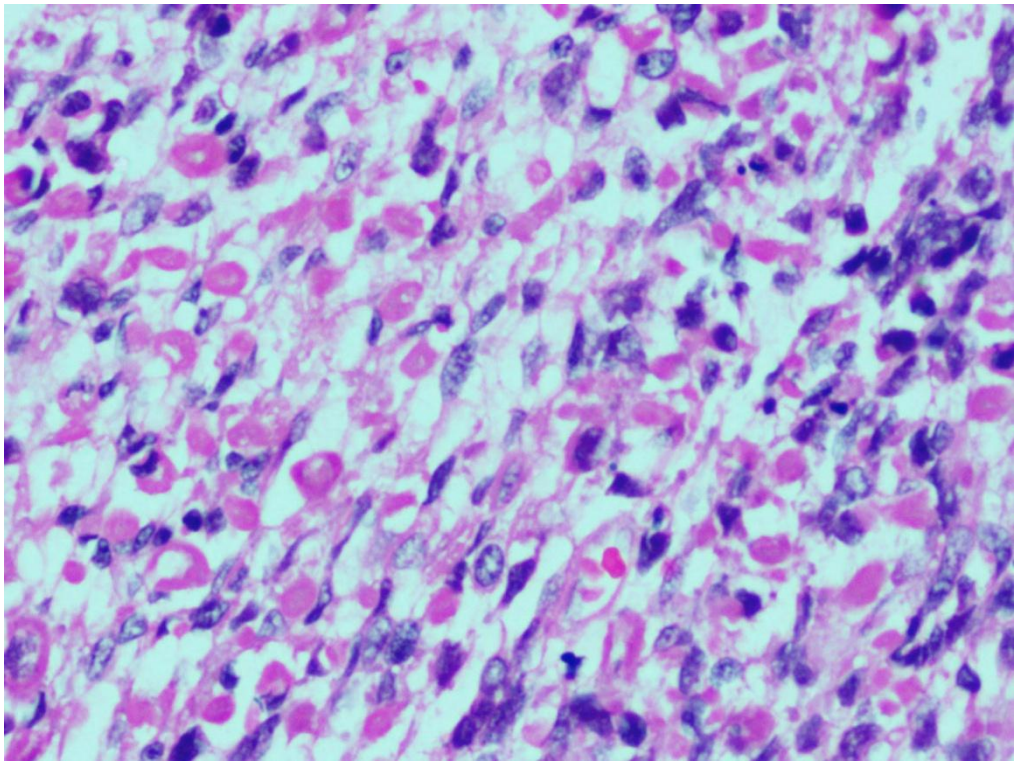
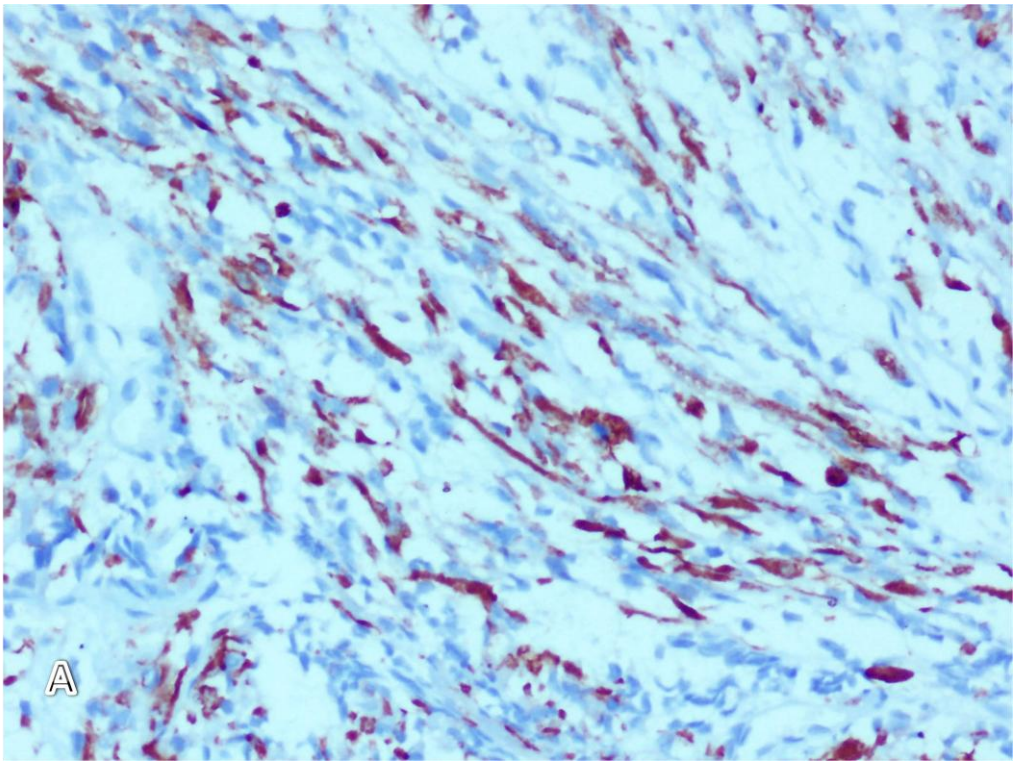


Figure 24(d): Rhabdomyoblasts in the Medulloblastoma with myogenic differentiation (H&E,x400)



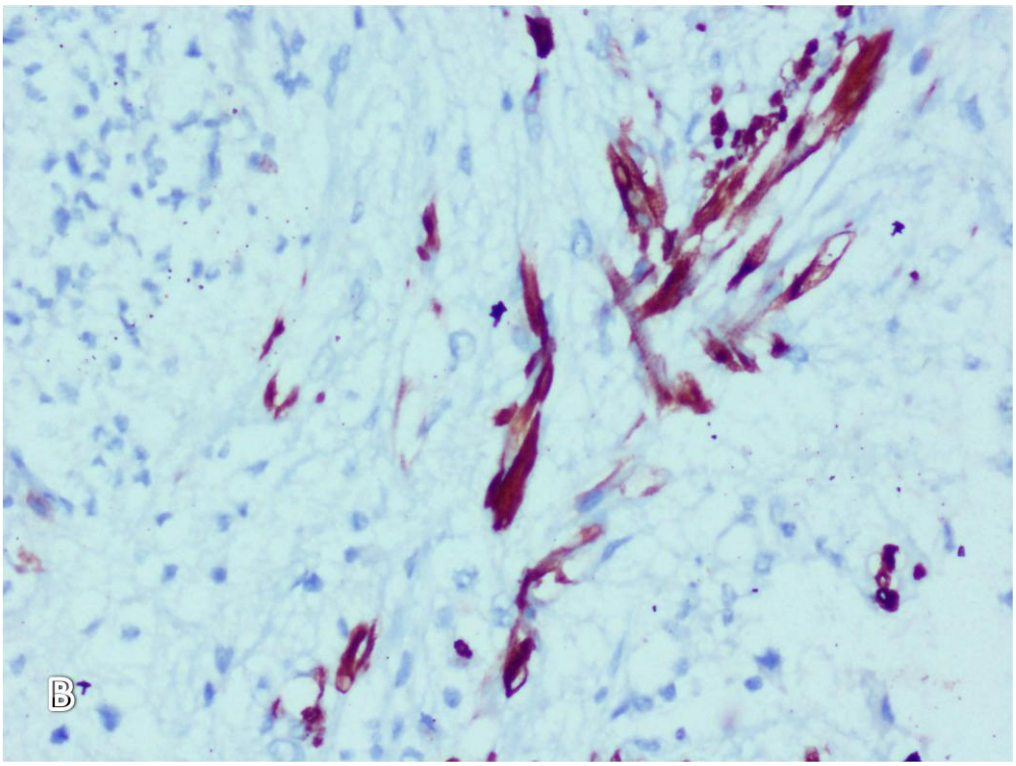


Figure 24(d):Desmin immunostaining of the myogenic component in medulloblastoma with myogenic differentiation.A)Low power magnification (x100) B)High power magnification(x400).

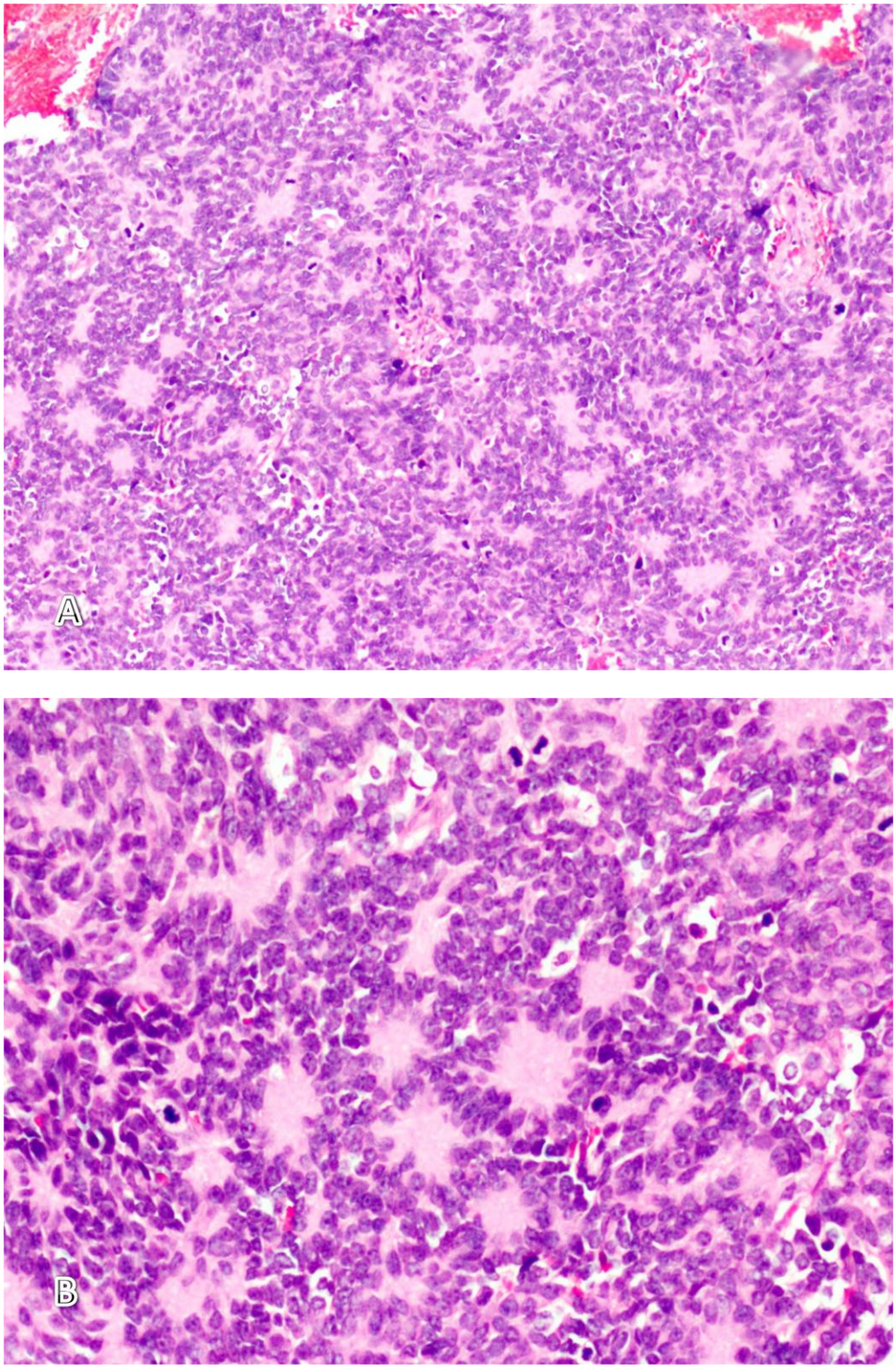


Figure 25(a&b): Classic Medulloblastoma with numerous Homer Wright rosettes

A)Low power magnification(H&E, x100) , B)High power magnification(H&E,x400)

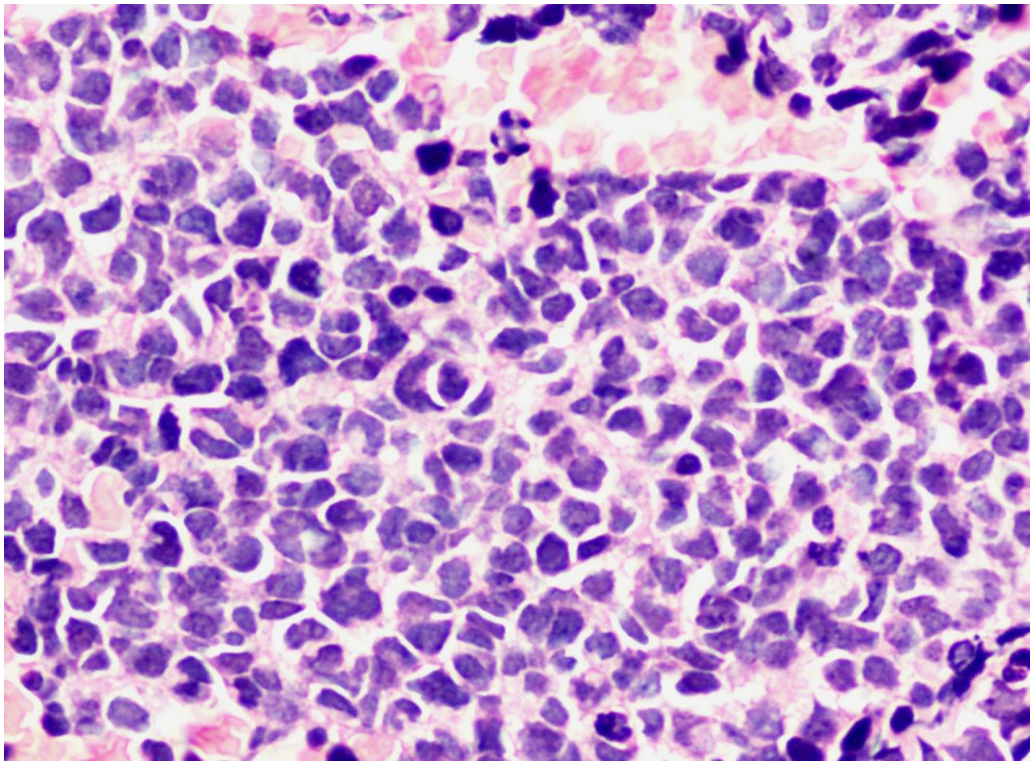


Figure 27: Nuclear moulding in an anaplastic medulloblastoma (H&E,x400)

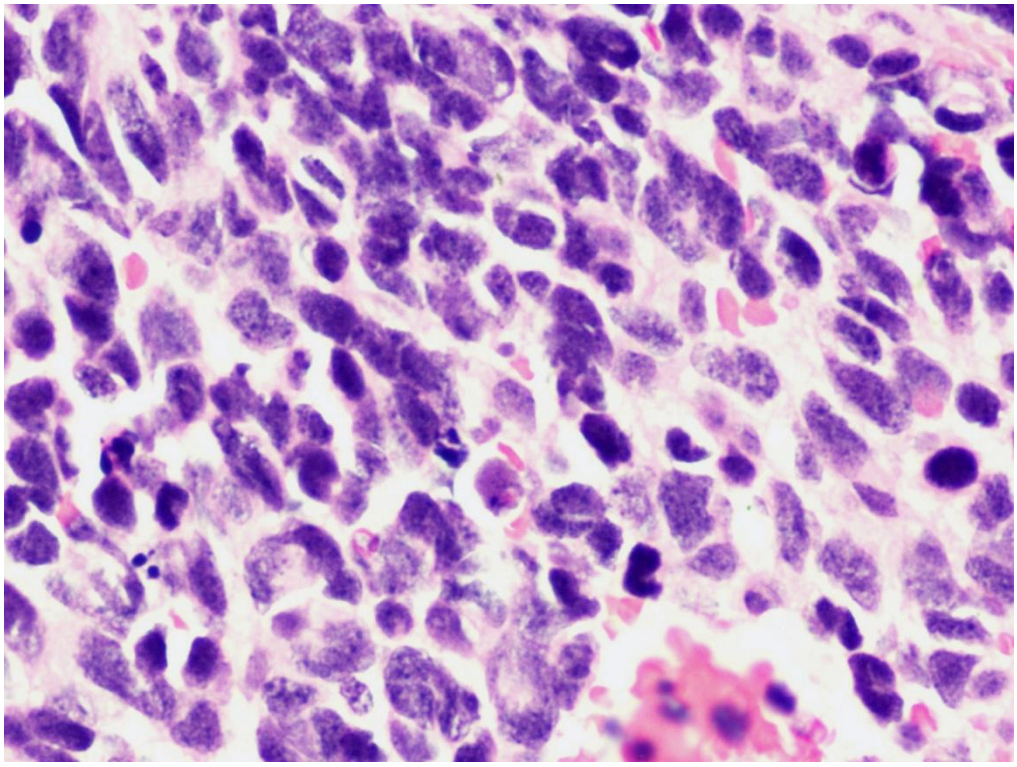


Figure 28: Cell to cell wrapping in an anaplastic medulloblastoma (H&E, x400)

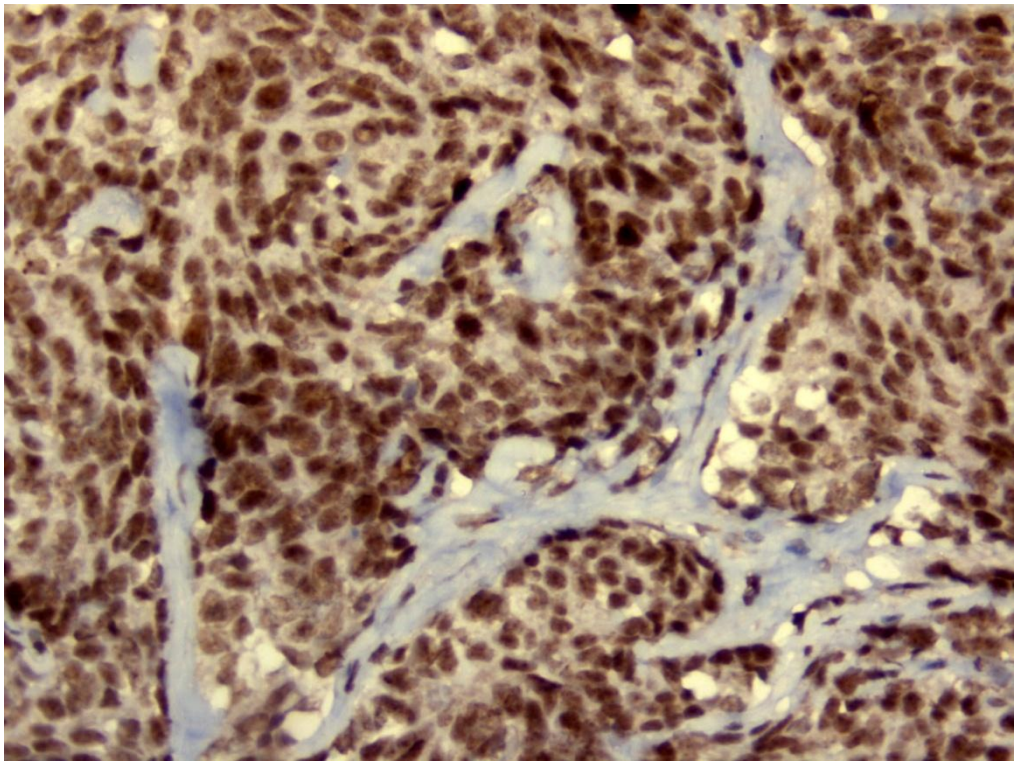


Figure 29(a):β catenin nuclear immunoreactivity in a classic variant of medulloblastoma(x400)

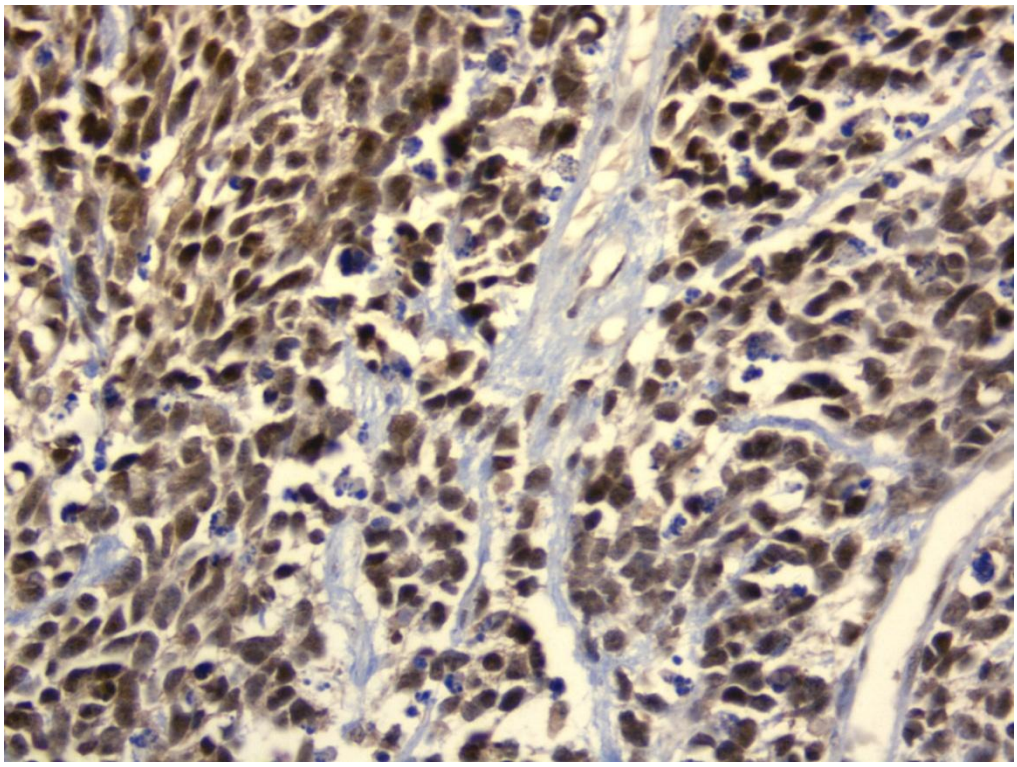


Figure 29(b):Both nuclear and cytoplasmic β catenin positivty in a classic variant of medulloblastoma (x400)

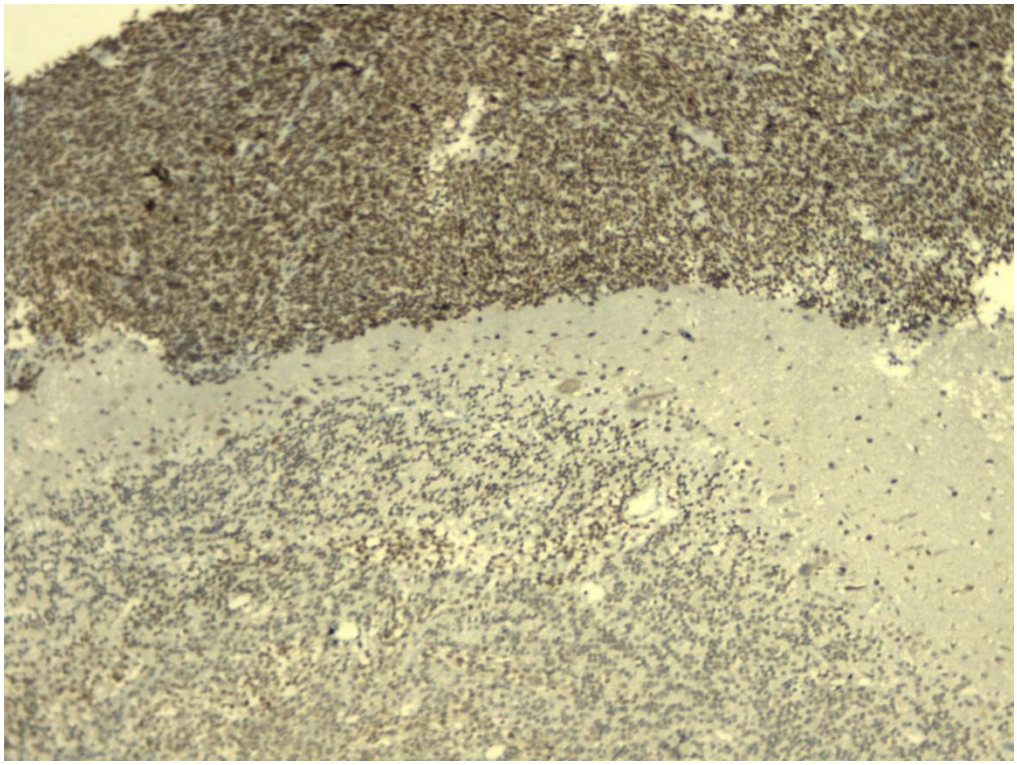


Figure 29(c) :β- catenin immunopositivity in the medulloblastoma with leptomeningeal invasion(x100)

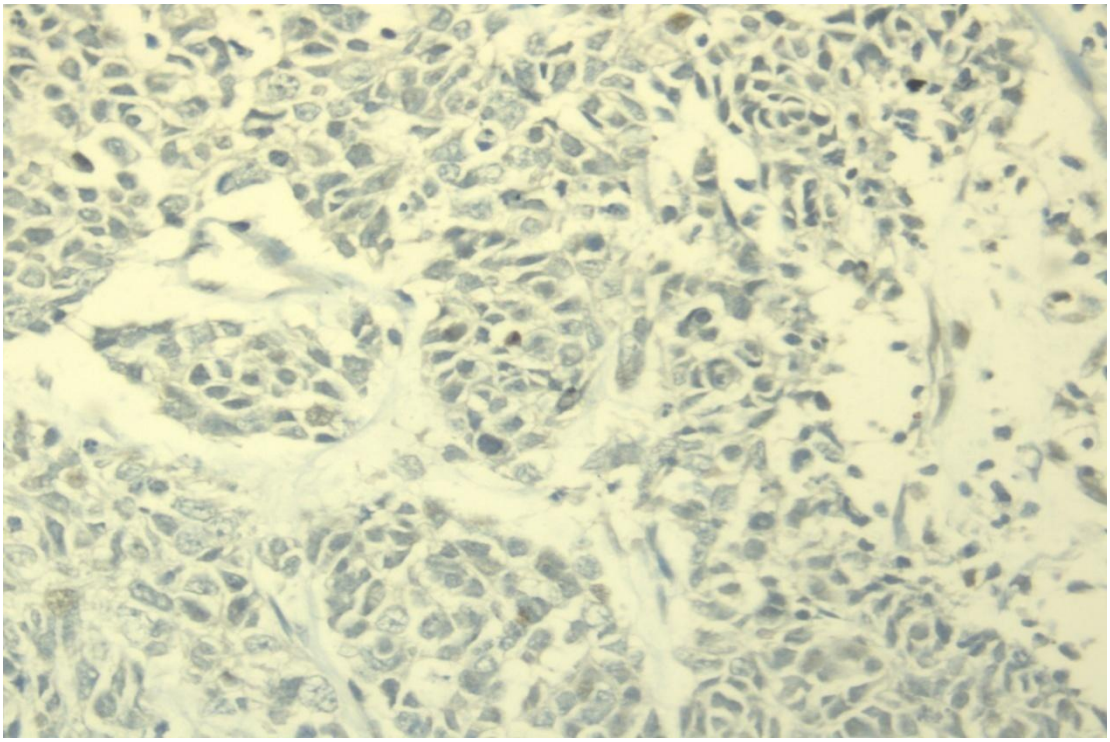


Figure 29(d): Medulloblastoma immunonegative for β-catenin (x400)

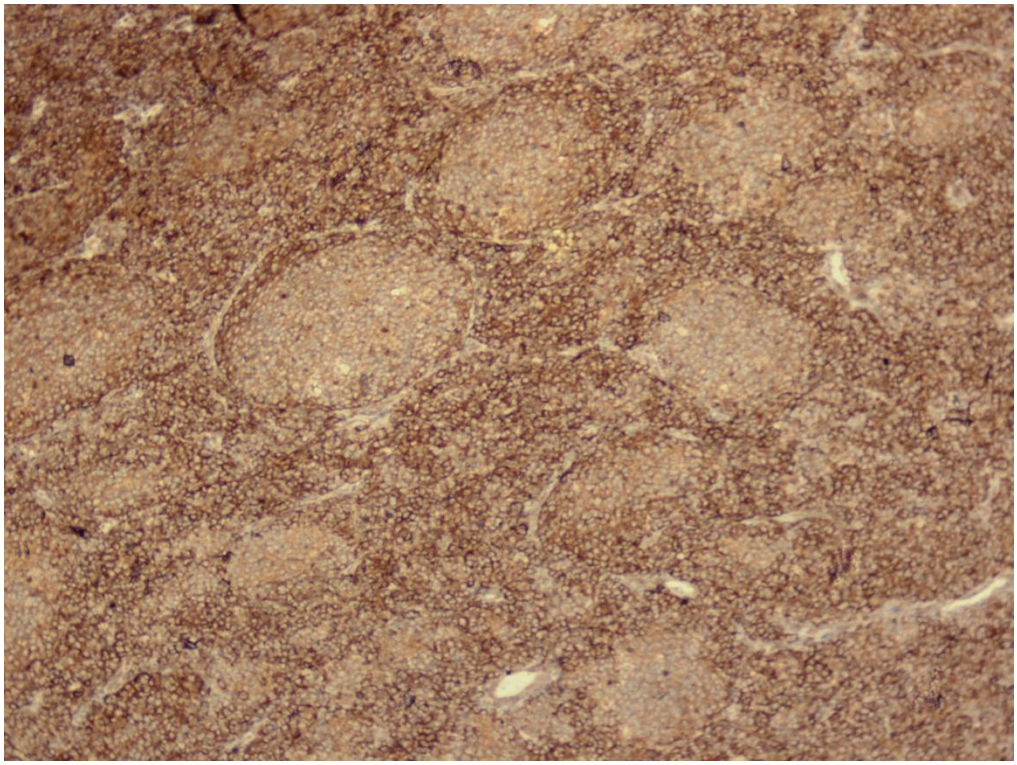


Figure 36 (a)..:GAB-1 immunopositivity in internodular areas of a desmoplastic medulloblastoma (x100)

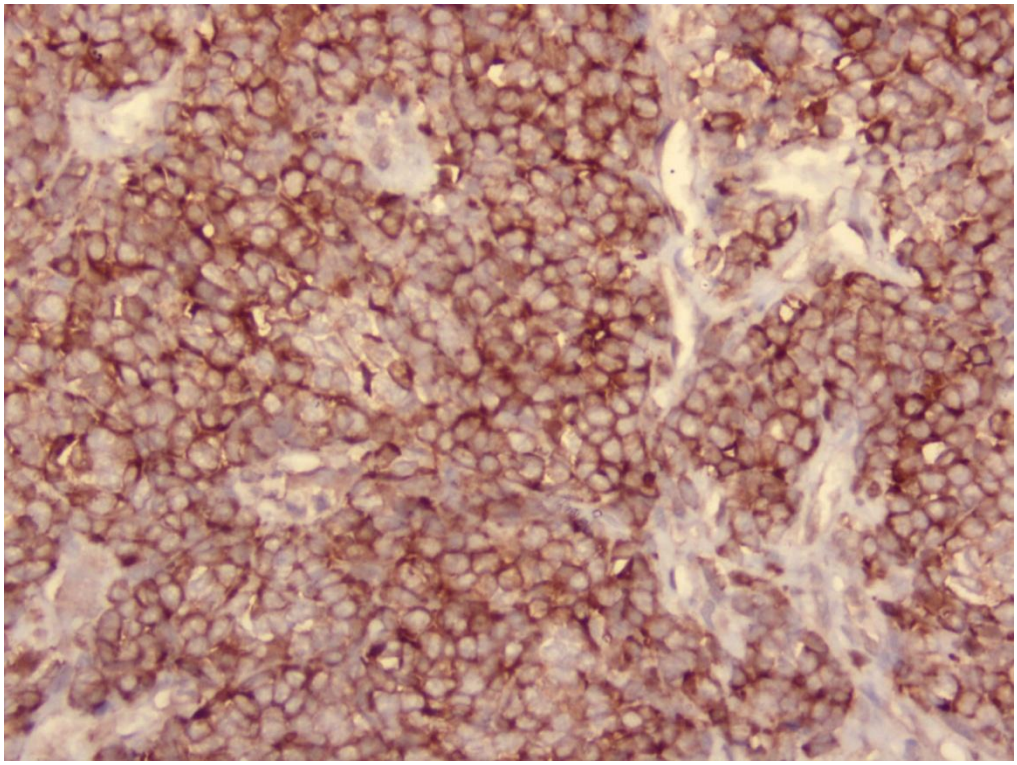


Figure 36(b):Cytoplasmic membrane positivity of GAB-1 in a desmoplastic medulloblastoma(x400)

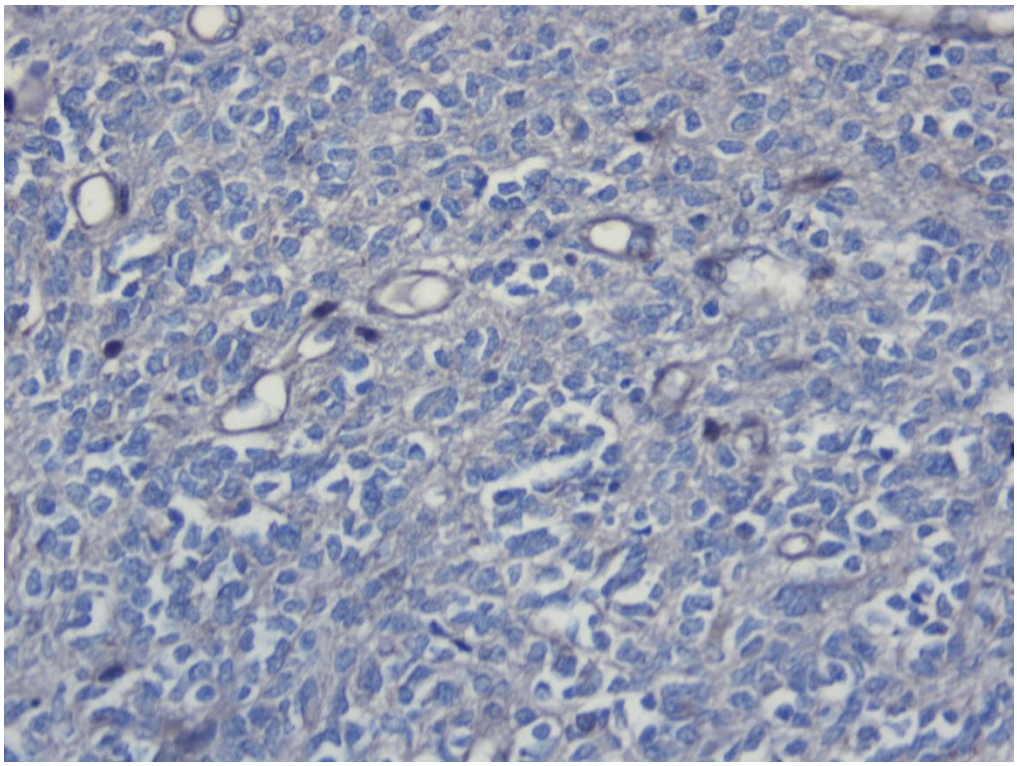


Figure 36(c):GAB-1 immunonegativity in a classic medulloblastoma(x400)

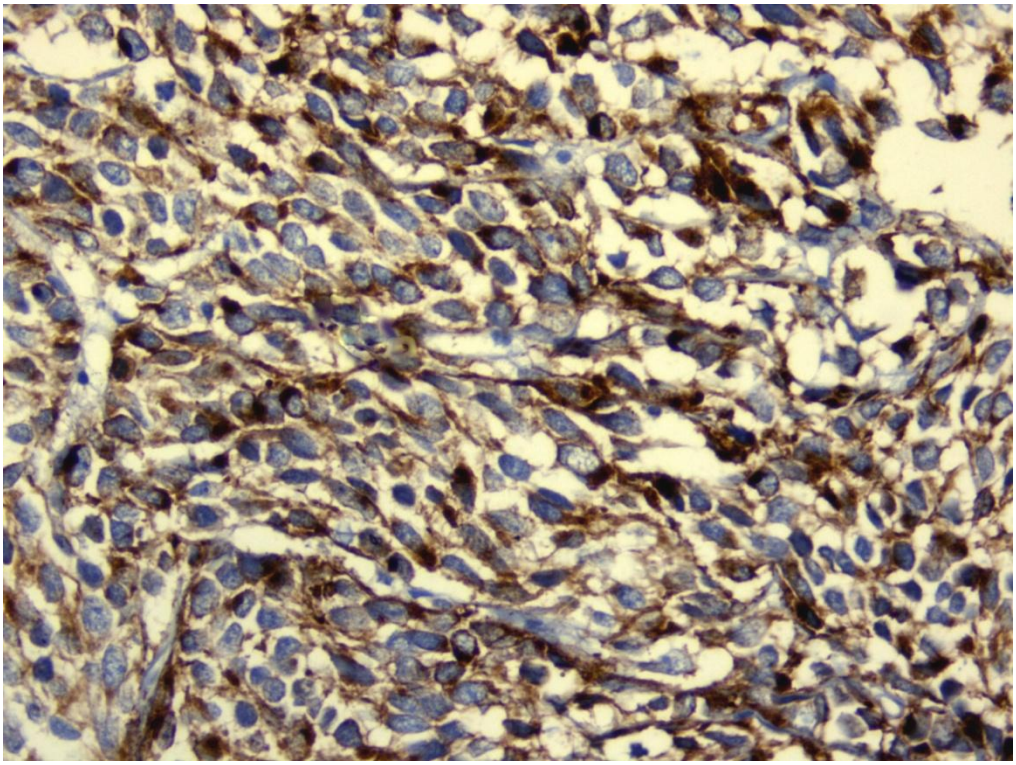


Figure 43: Medulloblastoma with immunoreactivity for NPR-3 (X400)